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TULAREMIA A SUMMARY OF CERTAIN ASPECTS OF THE DISEASE INCLUDING METHODS FOR EARLY DIAGNOSIS AND THE RESULTS OF SERUM TREATMENT IN 600 PATIENTS

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When this study was first projected it was recognized that tularemia is an extraordinarily variable disease, not only in its sources of infection and modes of transmission but also in its pathogenesis and clinical manifestations. Cases have been recorded in Cincinnati almost yearly since 1914 when Wherry and Lamb (1) first provided bacteriologic proof of the nature of the disease in man. A review of hospital and dispensary records reveals that durations of disease and of disability have varied from minima of one to two weeks to maxima of fourteen to fifteen months. Other measurable aspects of the disease varied similarly. A review of cases recorded in the literature indicated that similar variability occurred wherever the disease had been studied. It was therefore decided at the outset that statistical methods would be necessary to show quantitatively what effects, if any, serum treatment would have, and that an accurate appraisal of the value of the treatment could not be made without the accumulation of precise data from a series of serum treated patients and from a series of patients who had received only symptomatic treatment. Since the infection is characterized by prolonged morbidity and low mortality it seemed probable that comparative morbidity data would be more useful than mortality rate comparisons, at least during the first few years of the study, also that significant differences in various aspects of morbidity might become apparent before similar differences could be demonstrated in relation to mortality. Before the introduction of specific serum therapy treatment was entirely symptomatic, consisting of rest in bed, hot applications and analgesic

drugs for regional and general comfort, and incision and drainage of abscesses as necessary. Many drugs had been used empirically, such as quinine, iron salts, salicylates, mercurials, arsenicals and various dyes, but there is general agreement among close observers of the disease that none modified significantly either the natural course or the outcome of the infection. Since the patients who were to receive serum therapy would also have bed rest, local applications, and surgical intervention whenever any of these measures were indicated there seemed to be no objection to the establishment of a control group which would include patients who had received symptomatic treatment and patients who had received no treatment at all except rest in bed. If each group became large enough to be statistically stable any differences noted between the treated and control groups could be regarded properly as differences due only to serum treatment.

The aspects of the disease which were thought to be suitable for numerical expression, together with the definitions that were used in their determinations for both control and treated groups, are as follows. Duration of disease extended from the day of onset to the last day of symptoms referable to tularemia. Duration of disability extended from onset of illness to that time when it was possible to resume full and uninterrupted performance of the customary duties or activities. If suppurative adenitis occurred the termination of the duration of adenopathies was marked by the healing of the incision or sinus tract. In the absence of suppuration the duration of adenopathy extended from the day buboes appeared to (a) the day they disappeared or (b) to the time they reached a diameter less than one centimeter and were firm, not tender, painless, and continued to recede slowly from that time without subsequent enlargement. The febrile period included every day in which the oral temperature exceeded 37°C or 98.6°F . The bed ridden period included all days spent in bed at home or in the hospital. Complete epithelialization of ulcers marked the termination of duration of primary lesions. In the oculoglandular type this period terminated with healing of the conjunctival lesions. In the treated group the febrile period and the bed ridden period included all days of fever and bed rest that were caused by serum sickness. All instances of suppuration of buboes or of dermal lymphangitic nodules were recorded, and notation was

made if suppurations occurred before serum was administered. The occurrence of late recurrent adenitis, suppurative and non-suppurative, was also recorded for patients in each group.

With the aim of placing the collection of all data on a uniform basis I formulated a blank data sheet which could be used to record information from untreated as well as from serum treated patients, as shown

Name	Age	Occupation
Source of infection		Date of exposure
Date of onset of disease		
Primary lesion appeared (date)		Check clinical type
Location		Ultero-glandular
Date of healing of primary lesion		Oculo-glandular
		Glandular
		Typhoidal
Tularemia exanthem present?		
Location		
Duration		
Adenitis appeared	What nodes	
Dates of incisions, if any		
Date of healing of last incision		
Diameters of nodes on day serum was first given		
Same, 3 to 4 days after serum		
Total duration of visible and palpable adenitis		
Agglutination titers, with dates		
Same, after serum		
Bacterial skin test results, with dates		
Was antiserum skin test positive?		
Date (s) anti-serum was given		How given
Any immediate reaction?		Duration
What type of reaction		
Did serum sickness occur?	Severe?	Date of onset
Total duration		Urticaria?
Total febrile days from onset		
Days of fever after serum		
Total days remained in bed		
Date of resumption of normal activities		
Any late recurrent nodal swellings?		Onset
Duration		Suppurative?
Approximate or accurate date representing complete convalescence		
Total duration of disease		
Was illness complicated by other or secondary infections?		
Was patient suffering from other known illness when infected?		
Additional data		

FIG 1

in figure 1. In addition to providing spaces for the recording of the desired morbidity and mortality data opportunity was taken to acquire information about age, sex, occupation, source of infection, mode of transmission, incubation period, clinical type of the disease, the frequency, nature, and duration of the exanthem about which little seemed to be known, the stage of disease at which suppuration of buboes occurred, the priority of appearance of the primary lesions

or the buboes, the frequency, duration, and severity of serum sickness, primary or immediate reactions due to serum administration, and about the frequency, time of occurrence and outcome of late recurrent adenopathies concerning which very little accurate information was available. Records were also kept of the dates of performance and the results of serum agglutination tests, bacterial suspension skin tests, and immune serum skin tests. Notations were made of the dates and results of all of the relatively few cultural studies. Temperature records were condensed into daily maximal and minimal fever curves and transferred onto the backs of the sheets. On the sheets used for serum treated patients marginal notations were made of the day of disease upon which serum administration was begun, also the data pertaining to the serum that was used, its animal source, lot number, quantity and frequency of dosage.

The original plan was to make the comparative studies with data from untreated and treated cases from my own experience. However, after publication of the first papers which indicated that serum therapy modified favorably the course of the disease (2), there came an increasing number of requests from physicians who wished to give serum to their patients. The plan then adopted was that I would furnish serum in appropriate amounts, and a direction sheet for its proper use, to physicians having patients with verified diagnoses with the sole stipulation that in return the physicians would follow their patients to complete recovery and then send me the accurate information on the blank data sheets. I supplied a specially prepared suspension of killed *B. tularensis* for early diagnosis by means of the intradermal reaction and, in addition, performed numerous serum agglutination tests by the standard macroscopic method to aid in confirmation of clinical diagnoses. Whenever materials were sent to me, and frequently when patients were brought to my laboratory, I made guinea pig inoculations with various infected body fluids and isolated the infecting strains by culture of the heart blood at animal autopsy.

During each year that data on treated cases were accumulating, from my own cases, from cases in the Cincinnati region, and from physicians throughout the country, similar data were accumulated from untreated cases in the same geographic regions. Difficulty

was experienced in finding a sufficiently large number of control cases with accurately observed morbidity data to balance the growing body of treated case data obtained from physicians throughout the country. This deficit was made up by adding to the control series all of the case records presenting any quantitative information on the various aspects of morbidity that I could find recorded in the current literature. The duration of disease, of disability, of fever, and so forth, were often presented as approximate rather than as exact figures. All cases with only approximate figures were clearly marked so that if future need arose they could be culled out separately and subjected to comparative analysis with the precise data from the accurately observed control cases.

At the close of each year of study a statistical analysis was made in order to see what differences of significance might be observed between the control and treated groups, and also to learn whether or not any annual increment had caused a significant change from the constants derived in previous years. The results of two such comparisons have been reported (3). Although each successive comparative analysis had shown differences of a high order of significance between the control and treated groups the reported studies were open to the criticism that tularemia might not be quite the same disease in the Cincinnati region that it was elsewhere, that the manner in which the comparisons had been made would fail to show this, and that in this respect the control and treated groups might not be truly comparable. With so many different animal hosts involved as sources of infection, and especially with the passage of the infecting agent through insect and arthropod vectors, it is reasonable to expect that regional differences in virulence of the bacteria might occur, a fact already demonstrated for animals (4), and that these differences might be reflected in regional clinical variability. Although clinical descriptions of the disease from any other part of this country, even from European and Asiatic countries, do not reveal any differences in kind or in duration of clinical manifestations from those recorded in the Cincinnati region it had never been shown that the duration of disease, for example, had the same frequency distribution in one region as in any other.

With the exception of the previously stated regard for geographical

distribution the control series for the present study is composed of 518 unselected cases. The only criteria for admission to this group were verification of the diagnosis, absence of specific treatment, and available quantitative morbidity data. The component subgroups are 50 Cincinnati cases with accurately determined morbidity data, 170 cases with accurate data drawn from all over the United States except the Cincinnati region, and 298 cases with approximate data from anywhere in the country except the Cincinnati region. These subgroups were analyzed separately and compared with each other and with the whole group. There were no differences of statistical significance between the coefficient of variability of any subgroup and the coefficients of variability of any other subgroup, of any combination of subgroups, or of the entire group, respectively. There were also no significant differences between the means of any two of these subgroups. Hence it is clear that the duration of tularemia infection in the Cincinnati region varied between the same limits that obtained for this infection elsewhere in the country. The frequency distributions are shown graphically in figure 2. There were likewise no significant differences observed between these constants in relation to duration of disability, of adenopathies, of fever, or for the other selected aspects of morbidity. One may therefore safely conclude that throughout the period covered by these studies the salient clinical features of the disease varied between the same limits in the Cincinnati region that they did elsewhere in the United States, and that the control group is a proper standard of comparison for the treated group.

The treated group was composed of patients selected at random. The only criteria used for admission to this group were verification of the diagnosis, the administration of specific serum, and available quantitative data. After termination of the illness each data sheet was numbered serially and the numerical data for each case were transferred serially to a cumulative table with appropriate column headings. The 600 patients who form the present treated group were the first 600 to have their data so recorded. With the exception of a small number whose physicians could not be persuaded to return the data sheets every tularemia patient who received serum was included in the treated group. Since the morbidity data for this

group were derived from 81 patients observed and recorded by me, from 101 patients treated in the Cincinnati region and observed and recorded by other physicians, and from 391 patients treated in various parts of this country this group and its component subgroups were

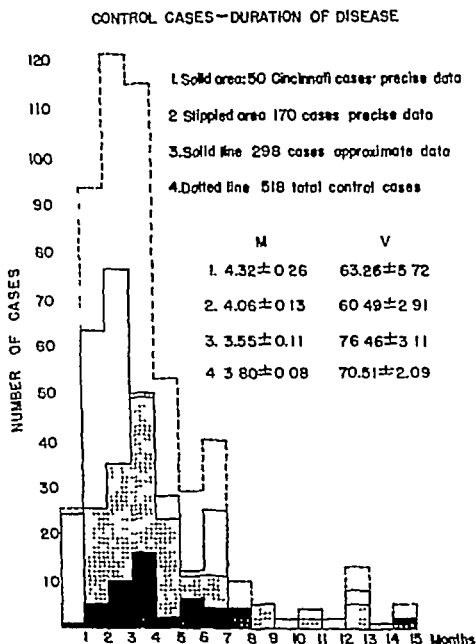


FIG 2 FREQUENCY DISTRIBUTION OF DURATION OF DISEASE OF THE CASES OF THE CONTROL GROUP AND OF ITS COMPONENT SUBGROUPS

The mean and the coefficient of variability, with their respective probable errors, are shown for each fractional group. There are no significant differences between any of these means nor between any of the coefficients of variability.

subjected to the same kind of comparative analyses that were applied to the control patient group. The frequency distributions for duration of disease of the subgroups and the entire group are shown in figure 3. None of the differences between the coefficient of variability of any subgroup and the coefficients of variability of any other sub-

group or combination of subgroups possess statistical significance. Hence the treated group is also composed of like subgroups. The

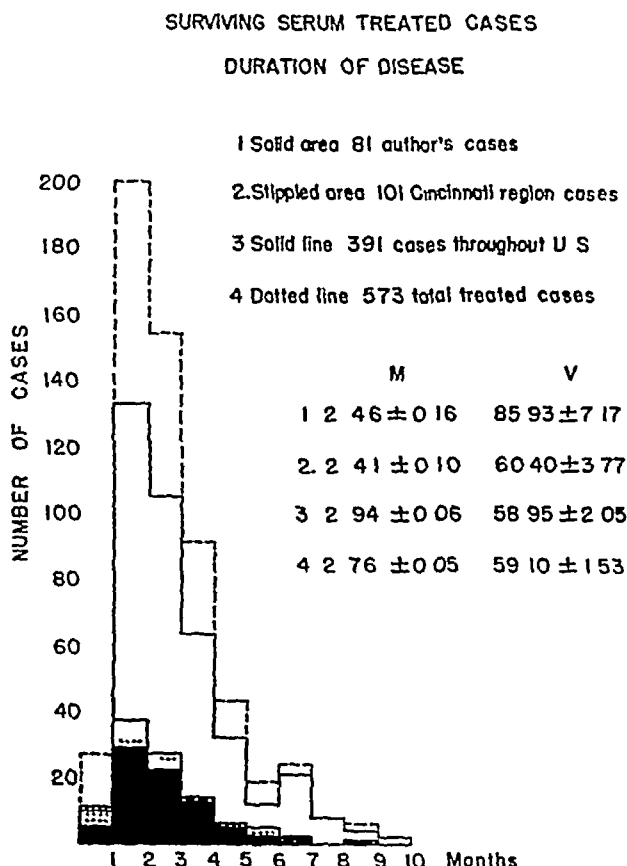


FIG 3 FREQUENCY DISTRIBUTION OF DURATION OF DISEASE OF THE SURVIVING CASES OF THE SERUM TREATED GROUP AND OF ITS COMPONENT SUBGROUPS

The mean and the coefficient of variability, with their respective probable errors, are shown for each fractional group. There are no significant differences between any of the coefficients of variability. There are no significant differences between any means except those of subgroups 2 and 3. Subgroup 2 contains the largest number of patients who were treated on or before the tenth day of disease. Subgroup 3 contains the largest number of patients who were treated after the twentieth day of disease. The mean day of disease upon which the patients in subgroup 2 received serum was the 16th day. That for the patients in subgroup 3 was the 30th day. The difference between these means shows a much higher degree of significance than the difference between the means for duration of disease. This difference in time of serum administration is sufficient to account for the significant difference in duration of disease observed between these subgroups.

shortened period of duration of disease due to serum treatment varied between the same limits in each of the subgroups regardless of the

geographic origins of the patients and quite without regard to whether the durations of disease were determined by one observer or by approximately 400 other observers. Similar insignificant differences were observed in relation to these constants for duration of disability, of fever, and to the other aspects of morbidity. Hence the changes in morbidity due to serum treatment of patients throughout the country at large were not significantly different from those observed in the Cincinnati region.

A review of the case histories of the patients showed an approximately equal representation in the control and in the treated groups of such factors as sources of infection, modes of transmission, age, sex, clinical types of the disease, pulmonary lesions, and the most frequent complications and sequelae. The one notable exception was the greater number of laboratory acquired cases of the typhoidal type in the control group. Cases that terminated fatally were excluded from the morbidity data computations for each group. The infrequently encountered case of chronic progressive tularemic infection was also excluded from each group since this study deals only with the effects of serum therapy in acute and subacute infections. Although a large proportion of the patients observed in the Cincinnati region and a lesser number of those observed elsewhere were studied roentgenographically for evidences of pulmonary lesions many of the diagnoses of pneumonia were based upon physical signs alone. Since chest films were not made routinely it is probable that the pneumonias that were found and recorded were in large part those which because of their size or location could be detected by physical examination.

The clinical classification of the disease used throughout this study is the one originally proposed by Francis (5). This classification is not favored by all students of the disease but, in my opinion, it does not compare unfavorably with others that have been suggested. The one proposed by Kavanagh (6), for example, makes no distinction between the glandular and the typhoidal types. Both are included in his cryptogenic type on the basis of the absence of primary lesions. In my opinion this is doubtful ~~antecedent~~ since the clinical appearance, diagnostic difficulties, and ~~course of disease differ markedly~~, even between these two types. The frequency of serious complications,

gravity of prognosis, and type fatality rates also differ markedly (7) This study will show that the indications for serum treatment, the responses to treatment, and the optimal dosages of serum differ notably between these types Since the typhoidal clinical type is outstandingly different from the others in so many important respects I think it would be advantageous if this form received separate designation in any clinical classification that may finally be adopted

SERUMS

The serums were prepared from goats and from a horse by prolonged series of subcutaneous inoculations with dense formaldehyde killed suspensions of virulent strains of *B tularensis* During the first year goat serums were made from two strains of high virulence, one recovered from the liver and spleen of a fatal human case at autopsy (8), the other recovered from the spinal fluid of a patient who died with tularemic meningitis (9) Since no reports of studies on antigenic differences among strains of this species could be found it was decided to anticipate this possibility by increasing the valency of the serums Strains that had been isolated in different regions were secured and additional ones were recovered by animal inoculations from patients in the Cincinnati region¹ From eight to twelve strains were used thereafter to inoculate the animals Virulence was maintained by occasional serial animal passage of each strain All serum was filtered through Berkefeld N candles Refinement and concentration by chemical methods were not attempted because of a prejudice approximating conviction that valuable antibodies are lost by such methods All serums were administered as whole serums During the first two years only goat serums were used During the next four years both goat and horse serums were used each year After two years there were two preparations of horse serum, that from my laboratory and the first to be prepared commercially During the last two years horse serum was used also in the lyophile form, dehydrated from the rapidly frozen state An

¹ Grateful acknowledgement is made to E Francis and C M Downs who kindly supplied strains that had been isolated in Virginia, South Carolina, the District of Columbia, and Kansas

advantageous concentration of this serum was effected by simply restoring only half the water that had previously been removed²

The usual mode of administration of serum was by the intravenous route. Many patients received it by intramuscular injection and most children were treated by subcutaneous injections.

Serum was first administered in small doses that varied from 10 to 25 cc. per patient. Later very much larger doses were given. About 100 patients were treated before a reasonably satisfactory scheme of dosage could be formulated. From this point on the average dose for an adult with disease of usual or average severity was 300 cc. The indications for the use of serum were at first the presence of disease, of fever, and of continuing disability. As experience grew it became necessary to modify and expand the indications for serum and the scheme of dosage, and these will be summarized later. Patients were treated at various stages of the illness, from the first to the 255th days of disease. This is shown graphically in figure 4. Ten patients who were believed to be in the dying state when first seen were given serum to see if moderate or large amounts would have any effect on the late septicemic stage of the infection. Dosage varied from 65 to 360 cc. of serum per patient. The morbidity and mortality data derived from the treated group include all patients regardless of the stage of the disease at which they were treated, and of the kind, amount, and mode of administration of serum that was given.

DIAGNOSIS

The macroscopic serum agglutination test was used for verification of diagnoses. The reliability of this test in tularemia approximates perfection. Although many thousands of cases have been studied in this and in other countries there is no report of a single failure of a patient to develop serum agglutinins unless death occurred during the first two weeks of the disease. Moreover, once agglutinins have been acquired as a result of infection they have not yet been found to disappear completely thereafter, even if tested for as long as thirty-

² The Mulford Biological Laboratories of the Sharp & Dohme Company kindly supplied antitularensis horse serum in both the liquid and lyophilic forms.

three years after recovery. The use of this one test therefore makes it possible to diagnose the disease, both in its acute phase and after recovery, with a degree of accuracy seldom encountered in other infectious diseases.

In its bubonic forms the disease is one of the easiest of infections to diagnose correctly from history and physical signs alone. The appearance of regional buboes with diameters of three to four centimeters without associated visible lymphangitis by the fourth to sixth days of a prostrating febrile disease in patients who give histories

TIME OF SERUM ADMINISTRATION TO THE 600 PATIENTS

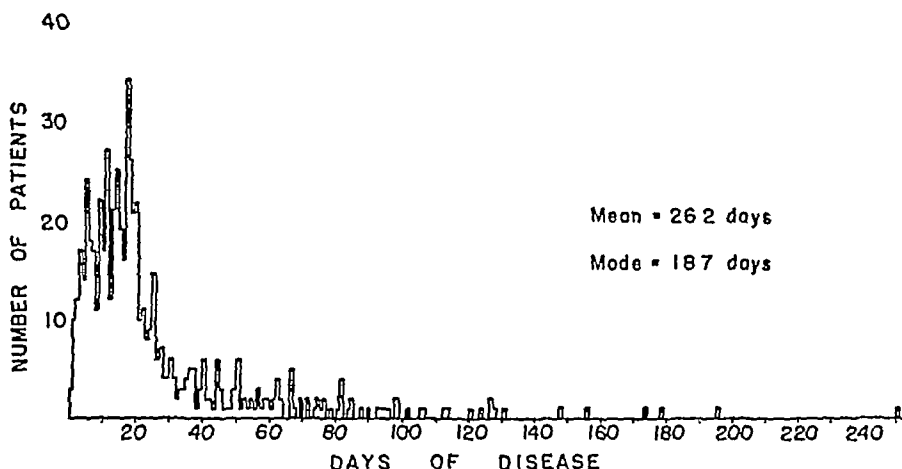


FIG 4 Approximately 57 per cent received treatment before the twentieth day of disease, and 76 per cent were treated before the thirtieth day of disease. The last patient to the right, shown as having been treated on the 250th day, was actually treated on the 255th day of disease.

of contacts with wild animals, ticks, or biting flies is very seldom duplicated by any other infection. Only one instance of diagnostic difficulty with reference to these clinical types was encountered. A digital chancre appeared on a physician a few days after he had killed wild rabbits. There was considerable fever, malaise, moderate prostration, and an unusual degree of regional lymph node enlargement. He was given antitularenses serum on the basis of these findings at a time when serum agglutinins could not have been expected to have developed. Tests made at that time, and subsequently, remained negative. No clinical improvement followed the serum

administration. Dark field examinations revealed the infecting spirochetes and set the diagnosis right. Since tularemia was not present this patient was excluded from the treated group.

The typhoidal clinical type often presented great difficulty in diagnosis and it was always necessary to resort to laboratory aids for confirmation. More cases of this type would be diagnosed correctly, and earlier, if the possibility of tularemia were borne in mind and if diagnostic tests were applied at once and repeated every four or five days, especially for patients with unexplained fevers and atypical pneumonias that occur during the rabbit, squirrel, and quail hunting seasons, and during the periods of greatest activity of ticks and blood sucking flies. Early and accurate diagnosis is especially desirable since the typhoidal type has a mortality risk four times that of the other types (7) and because it will be shown that patients with this type respond especially well to early serum treatment.

Although the serum agglutination test is extremely reliable the well established knowledge that serum agglutinins never appear during the first week of disease, and occasionally may not appear until the third week or, rarely, until the fourth week, made it unsuitable as a test for early diagnosis. Nevertheless the inevitability of eventual appearance of agglutinins makes this test an ideal standard with which the results of other diagnostic aids can be compared.

Since the introduction of the bacterial suspension intradermal test reaction as an aid in early diagnosis (10) information has been accumulated on the reliability of this test. The tests were performed by injecting intracutaneously enough of a specially prepared *B tularensis* suspension, usually about 0.05 cc., to make a skin wheal 5 mm. in diameter. Because of the previously determined high degree of specificity of the reaction, by correlation with agglutination tests, control tests were seldom used during recent years. When control tests are desired they should be made with 0.05 cc. of physiologic salt solution containing 0.5 per cent phenol. Falsely positive reactions have not yet been observed, even in brucellosis patients whose serums cross agglutinated *B tularensis*. A positive reaction has the appearance of a positive tuberculin test and, like the latter, usually requires forty-eight hours for its development. No delayed reactions, appearing after the forty-eighth hour, have been noted.

The degree of sensitivity to specific vaccines that is acquired by tularemia patients is often extremely high. During the early period of development of the skin test suspension a few patients were tested with highly diluted vaccines made in the usual ways, with heat killed or formaldehyde killed bacteria. All of these patients suffered large, necrosing local reactions and many of them had hyperpyrexia with serious focal reactions such as rapid accumulation of serous effusions in the pleural cavities, rapid and extensive enlargement of pneumonic consolidations and, in one case, a complete diplegia at the tenth thoracic cord segment that lasted for seven hours in a patient who became comatose to semicomatose for nine hours. Since it seemed impossible by trials with further dilution to make these vaccines reliably skin reactive and at the same time incapable of provoking such alarming constitutional reactions it seemed necessary to resort to chemical methods to diminish their reactivity. A suitable vaccine for skin test purposes was prepared by oxidizing four pooled virulent strains with nitrous acid (10). This suspension gave reliable diagnostic reactions of reasonable size, seldom produced necrosis, and very rarely provoked mild constitutional reactions. Its activity gradually diminished over a period of about one year and therefore it should not be relied upon as an accurate diagnostic aid if it is more than one year old. The experience with other types of vaccines justifies a warning that there may be danger for certain patients if the standard formaldehyde killed suspensions which are made for use in the agglutination test are diluted and used for skin test purposes. The great majority of patients showed positive reactions when they were tested on any day during the first week of illness or thereafter, and of 428 patients who were tested on or after the eighth day of disease only three failed to give positive responses to the first test that was made. Most of these patients had had positive agglutination tests before the skin tests were made. The chief advantage claimed for the skin test is that it will usually confirm the diagnosis during the first week of disease when serum agglutinins are always absent. Of 108 patients who were tested during the first six days of disease eight failed to give positive reactions to the first tests that were made. Repetition of the tests at intervals of forty-eight hours in these eight patients resulted in positive skin

reactions before serum agglutinins appeared in every patient but one. Patients in the dying state did not usually react to the skin test. The defects of the test were those of intradermal tests in general, but this one appeared to be as reliable as any other. If the negative responses to tests made during the first week of disease were followed by repetition of the test at forty-eight hour intervals as often as necessary the accuracy of this test for early confirmation of the diagnosis very closely approximated that of the agglutination test in later stages of the disease.

Observations were also continued on the use of specific immune serum as a diagnostic intradermal test (11). The materials used were antitularensis goat or horse serums and strictly normal serums from these same animal species, all in dilution of 1:10 in phenolized salt solution. Goat serums were found to be preferable to horse serums because normal goat serum seldom produced an erythematous reaction in the skin of normal individuals and because natural or artificial sensitization to goat serum proteins was encountered much less frequently than sensitization to horse proteins. The tests were performed by injecting intradermally 0.04 cc. of the diluted immune serum at one site and the same amount of diluted normal serum at another site. It is imperative that the control test be made every time the immune serum is used in order to exclude the possibility of previous serum sensitization. Positive reactions appeared almost immediately in most cases, seldom later than the third minute and never later than the sixth minute after injection. The reaction consisted of two phases, an immediate areola of erythema and then an enlargement of the central wheal to two to three times its original diameter. A positive test consists of a reaction of this nature at the site of injection of the immune serum accompanied by a complete absence of reaction from the normal serum. The reactions should be compared and read fifteen minutes from the time the injections are made. The reaction is extremely delicate and syringes that have once been used for immune serum must never be used for the normal serum unless they have been thoroughly cleaned by soaking in acid bichromate cleaning solution.

This test has become positive as early as eight hours after the initial chill. In the 108 patients who were tested during the first

six days of disease this test failed to become positive in eight. These were the same eight patients who failed to show positive reactions to the first bacterial skin tests which were applied simultaneously. Repetition of the serum test brought out positive reactions at the same times that the bacterial tests became positive, with allowance made for the forty-eight hour difference between their appearance times. In 360 patients tested by all three methods there was complete agreement between the results of the serum agglutination test, the bacterial skin test, and the antiserum skin test.³ The antiserum test is not recommended for general diagnostic use until proper test and control serums can be made available.⁴

The simplified phagocytic test devised by Huddleson and his associates (12) for the study of brucellosis was applied to a small number of tularemia patients, using suspensions of both killed and living *B. tularensis*. Opsonins always appeared simultaneously with serum agglutinins, never before, and hence the test was of no value in early diagnosis. By testing a small number of recovered immunes it was noted that opsonins, like agglutinins, persisted for at least twenty years after recovery from the disease.

Errors in early diagnosis were usually traceable to failures to elicit histories of exposure, to misapprehension concerning the variability of the clinical aspects of the disease, or to failures to make repeated applications of diagnostic tests.

PATHOGENESIS, CLINICAL COURSE, AND PROGNOSIS OF THE UNTREATED DISEASE

The account of the disease which follows is based upon a study of reported cases and necropsies, on the extensive researches conducted by Francis and his associates in the United States Public Health

³ Attention is directed to a report, published since this paper was written, which records a much less favorable experience with the antiserum intradermal test as a diagnostic aid. See Friedewald, W. F., and Hunt, G. A. The Diagnosis of Tularemia. *Am. J. Med. Sci.* 197, 493 (April), 1939.

⁴ By arrangement with the Coordinating Committee of the American Public Health Association I have agreed to furnish immune serum and normal serum, also bacterial test suspension, to physicians for diagnostic use with the understanding that the results of all tests made will be returned to me for compilation and study in regard to continued reliability of these diagnostic tests.

Service on many aspects of the disease in man and in animals, including the recent monograph on its pathology (13), and on the information and experience with surviving and necropsied fatal cases that I have accumulated during the past ten years

A primary lesion usually develops at the site of inoculation. At first a small red papule, it soon enlarges and ulcerates, liberates a necrotic core and leaves an indolent ulcer with raised edges, a ragged floor, and a punched-out appearance. Most occur on the skin of the upper extremities, face, or in the conjunctivae, but they also occur in the pharyngeal cavity and in the nares. They may be single or multiple. In about ten per cent of cases the bacteria penetrate the skin without producing a primary lesion.

There is usually an initial bacteremia, with origin at the site of primary invasion, which commonly persists for not longer than a week or ten days. In a few individuals who, like most rodents, have little or no natural resistance the initial blood stream invasion is either a septicemia from its onset or else soon becomes a septicemia which results in early deaths from the fourth to the eleventh or twelfth days of disease.

Bacteria invade the sweat glands and also both the superficial and deep lymphatic vessels at the inoculation site. Extension of infection up the superficial lymphatics results in the formation of dermal lymphangitic nodules along their courses, very similar in appearance to those which occur in sporotrichosis. Nodular lymphangitis occurs in about seven per cent of the cases that develop lymphadenitis at any site. Invasion of the deeper lymphatics results in regional lymphadenitis and bubo formation which is characteristic of more than ninety per cent of human cases. Lymphangitic invasion is not a constant feature. In about eight per cent of human cases there occurs no bubo formation. In such cases there are very seldom any discoverable primary lesions.

The transitory initial bacteremia commonly results in the formation of a small number of widely scattered focal necroses in the spleen, liver, lungs, in lymph nodes other than the regional nodes, and occasionally in other organs. The small pulmonary foci may initiate areas of bronchopneumonia or may develop into discrete nodules that enlarge, become caseous, and occasionally liquefy with resultant

abscess formation. In some cases a heavy pulmonary dispersion produces diffuse miliary focal necroses which simulate the appearance of pulmonary miliary tuberculosis. The development of the occasional focal lesions in other tissues proceeds similarly. In surviving cases healing occurs with scar formation. In patients who have died of causes other than tularemia the abdominal lymph nodes may contain liquid pus as late as a month after the onset of disease. When antibodies appear in the blood the bacteremia disappears in surviving patients and from this stage onwards there is probably no formation of fresh lesions except by lymphatic extensions. Since most deaths occur at a later stage, and since there is cultural and necropsy evidence of a septicemia for five or six days prior to death, it is necessary to postulate a second blood stream invasion to account for the necrotic findings in the great majority of fatal cases. This second bacteremia seems to be invariably a fatal septicemia with origin in any of the previously established lymphogenous or hematogenous foci of necrosis, by ulceration of blood vessel walls or by lymphatic access to the blood circulation. It results in the production of myriads of miliary and submiliary foci of necrosis, chiefly in the spleen, liver, lungs, lymph nodes, and serosae, and to a lesser extent in the intestines, suprarenal glands, kidneys, meninges, brain and other organs. As in miliary tuberculosis, the bacterial dispersion may be through both the pulmonary and the aortic circulations or it may be largely limited to either circuit.

The incubation period varies from one to ten days but is usually two to five days. Prodromal symptoms are rare and extremely few patients experience a feeling of general uneasiness, vague gastric disturbance, slight vertigo, or mild localized pains at the sites of future development of primary lesions or of buboes prior to the abrupt onset.

The onset is characteristically sudden, with headache, fever, chills, nausea, chills, profuse sweats, aching bodily pains, nausea, vomiting, and marked prostration. Diarrhea and colicky pains may occur at the onset. The primary lesions and enlarged lymph nodes are usually present within forty-eight hours from the onset, and may be contemporaneous with it. Visible lymphangitic streaks occur only when there has been secondary infection of a primary lesion.

Regional nodes are involved first and are often the only ones to be visibly enlarged. The combination of a unilateral primary lesion with bilateral buboes is not uncommon. Nodular lymphangitis usually appears on the upper extremities. Nodules are usually multiple, their numbers ranging from two to thirty. The primary lesions heal very slowly with scar formation. Their average duration in 455 cases was thirty-nine days.

Cough is frequently an early and transient symptom. It usually disappears by the end of the second week. Although roentgenologic studies (14, 15, 16) indicate that minor degrees of bronchopulmonary change occur in ninety per cent of all cases only about eighteen per cent of patients develop pneumonic lesions large enough to be discovered by physical signs or to be clinically significant. Bronchopneumonic consolidations may be solitary or multiple, unilateral or bilateral, and with or without accompanying pleurisy which may be dry or accompanied by effusion. Consolidations may develop in any lobe but they appear most frequently in the hilar regions with extension into the lower lobes. By extension or confluence they may produce the physical signs of a lobar distribution. Pneumonia is regarded more as an integral part of the disease than as a complication. Pleurisy may occur without detectable underlying pneumonia by either physical or roentgenologic examinations.

Fever is always present and usually reaches a level of 39 to 40°C (102.2 to 104°F). There is usually an initial rise, a remission, and a secondary rise. The initial rise commonly lasts from one to four days. Then occurs a period of remission which may last from one to five days, followed by a secondary rise to the initial height which persists for one to two weeks, after which there is a gradual decline to normal. The entire febrile period usually lasts from two to four weeks. The febrile remission is accompanied by an amelioration of all symptoms which leads many to anticipate a prompt recovery, but the symptoms return with the secondary rise of temperature. In the presence of persisting pneumonic lesions, of suppurating lymph nodes, or of various complications there may be continuation of fever for several additional weeks or even for months. In 495 cases the average duration of fever was thirty-one days. The fever is characteristically of the daily remittent, or "septic" type, with wide

fluctuations When septicemia occurs the fever usually becomes non remittent at a high level This change may occur over a twenty-four hour period but often it occurs quite abruptly with the advent of the septicemia

In addition to the change in the character of the fever curve the onset of septicemia is usually marked by the coincident appearance of delirium, confusion, or stupor, with rapid development to a semi-comatose condition These earliest changes are soon followed by progressive enlargement of the spleen and liver, often with increasing jaundice, deepening coma and signs of meningeal or focal cerebral lesions, the development of new pneumonic lesions or the confluence of preexisting ones, or the appearance of the physical findings which are usually associated with pulmonary miliary tuberculosis, tympanites, abdominal pains and diarrhea, the urinary findings of acute nephritis or of intense acute nephrosis, and signs of progressive involvement of the pleurae, pericardium and peritoneum Weakness, loss of weight, and recurring chills and sweats are characteristic of the active stage of the disease Cases that are mild throughout the entire course are seldom seen In about half of all patients, however, the symptoms that return concurrently with the secondary rise in temperature are milder than those which occurred at the onset, and these individuals are bed-ridden for only a week or two and soon become ambulatory A rare individual may continue to work throughout the entire course of the illness Inapparent or subclinical tularemic infections have never been discovered

The most frequent complication is suppuration of the buboes which occurs in more than half of all cases and most frequently during the first five months from onset The frequency of suppuration of the dermal lymphangitic nodules is about the same, perhaps slightly greater, than the suppuration frequency of lymph nodes An unusual feature of the disease is reenlargement of lymph nodes after they have receded to normal dimensions Secondary enlargement of nodes has been observed from the third month to the third year after apparently complete recovery At least sixty per cent of the late recurrent adenopathies progress to suppuration The pus of those that break down after the sixth month of disease is always sterile as judged by cultures and animal inoculations, and it contains numerous giant

cells of the foreign body type. The pus of nodes that suppurate during the first five months of disease usually contains viable *B tularensis*. Secondary infection of primary lesions frequently results in continuous or intermittent suppurative processes that persist for weeks or months.

The consequences of tularemic pneumonia are resolution or organization of the exudate, pulmonary abscess, empyema, extensive pulmonary and pleural fibrosis, and bronchiectasis. Pericarditis, hepatitis, enteritis, meningitis, encephalitis, and thrombophlebitis have been observed. Less frequent complications are appendicitis, and general peritonitis with ascites. With the exception of pericarditis, patients who had normal hearts at the time infection was acquired have not had complications or sequelae referable to the heart. However, in the presence of pre-existing rheumatic or vascular heart disease attacks of angina pectoris, coronary occlusions, and transient periods of complete heart block have occurred, sometimes during the active febrile stage of the infection but more frequently during early convalescence. Subacute bacterial endocarditis due to the usual streptococci has occurred infrequently as a coincident infection. Endocarditis due to *B tularensis* has not yet been demonstrated.

Relapses or recrudescences have occurred from eight months to two years after the onset. Although most have been solitary and of brief duration, instances of repeated relapses have been recorded (17). The frequency of occurrence of relapse is not known, but it is probably low. An apparently uncommon but exceedingly disabling sequel is the persistence of the infection in a chronic progressive form. Little is known at present concerning its frequency of occurrence, pathogenesis, duration, or outcome. In rare cases symptoms have been practically continuous, more frequently the condition is that of persistently recurring relapses, each marked by fever, sweats, malaise, profound weakness, and mental depression. Painful, tender enlargements of lymph nodes may appear at the onset of each relapse but this is not a constant feature.

The prognosis in regard to survival in untreated cases is good. Although man is very susceptible most patients show a high degree of natural resistance to the infection, and the case fatality rate is

probably not above six per cent. The supervention of septicemia significantly alters the prognosis. There is no record of survival of an untreated patient who had tularemic septicemia, and life seldom persists for more than six days after its onset. The prognosis is less favorable if pneumonia is present, especially if the consolidated areas are large, and it becomes worse if delirium and confusion supervene. The mortality risk of the typhoidal type is four times greater than that of the other clinical types. The prognosis is bad for patients with rheumatic or coronary disease. Most deaths occur at the beginning of the third week of disease, and they occur most frequently as a result of septicemia, much less frequently from toxemia and heart failure.

Although the duration of disease varies from a week to fifteen months, in most cases it lasts about four months. Successive supuration of lymph nodes is the most frequent cause of protracted illness. Other common causes for prolongation of disease are progressive or migratory pneumonic lesions and their sequelae, and the persistence of infection in the serous cavities. Convalescence is usually slow. It is rare for a patient to be at work again at the end of a month. Usually the second month is spent lying about the house owing to weakness on exertion, and during the third month only half-time work is performed. Recovery from attack confers a permanent immunity.

Seasonal incidence of human cases results from seasonal variation in the three chief sources of infection, the handling of wild rabbits, the activity of biting flies, and the feeding periods of ticks. East of the Mississippi river, or wherever State game laws protect rabbits for the greater part of the year, the great majority of cases occurs during the hunting season, usually in November and December. In the West, where the jack rabbit is unprotected, cases acquired from the rabbit occur throughout the year. The period of greatest activity of biting flies is from June to September, and in regions where the fly is an important vector there is a high incidence of cases during the summer months. In the Northwest tick-borne infections occur from March to August, the period of greatest activity of the Rocky Mountain and Western wood ticks. In the southern and eastern States tick-borne infections occur from January to October, mostly

from April to the end of July, the period of greatest activity of the common dog tick or wood tick. Due to overlapping and variation in these influences cases occur in the United States in every month of the year

Annual prevalence in any one geographic region varies from year to year. There is a positive correlation between the number of human cases in a given locality and the regional prevalence of the infection in animal hosts and in arthropod or insect vectors for that year. The nature of the onset with respect to certain predominant early symptoms may also vary from year to year. For example, in 1933 and 1934 the great majority of patients in the Cincinnati region had bronchitis at the onset, and cough was a prominent early symptom. In 1936 about one third of all patients had onsets characterized by abdominal pain, nausea, vomiting, and diarrhea. Cough was then an infrequent complaint and bronchitic rales were seldom noted. Prior to this year initial abdominal symptoms had been infrequent, the record showing only sixteen such cases in about 450 patients recorded during the previous five years. In the fall of 1936 there occurred a large endemic outbreak in the Cincinnati region with about 140 cases in the six week period between November 15 and December 31. Similar increases over the usual annual number of cases were noted that year as far north as Dayton, Ohio, and in southeastern Indiana and northern Kentucky. In the entire regional outbreak early abdominal symptoms occurred with unusual frequency. Tympanites with great abdominal distension was common, and pain was often very severe. These patients were profoundly intoxicated and their suffering was much greater than that usually experienced in the initial acute phase. Since most of these patients had the usual ulceroglandular clinical type with primary lesions on the hands it seemed most unlikely that this unusual type of onset could have been due to the ingestion of infected food insufficiently cooked.

THE SERUM TREATED GROUP

Epidemiology

With few exceptions the sources of infection and modes of transmission did not differ from those already well established by others

Since most cases occurred east of the Mississippi river the great majority resulted from contact with wild rabbits. Three cases apparently occurred as a result of dressing domestic chickens. In each instance every other possible source of infection was carefully excluded, and in each case the origin of the fowl was traced to rural or subrural poultry farms where wild rabbits were abundant.⁵ It is well known that the rabbit tick, *H. leporis-palustris*, which is chiefly responsible for the perpetuation of the disease in the rabbit population, will accept gallinaceous birds as hosts. The initial presumption was that the chickens might have acquired the infection from infected rabbit ticks. Poultry dealers frequently sell wild rabbits in season. Hands contaminated by dressing rabbits may transfer bacteria mechanically to fowl as they are dressed. Another possible mode of transfer, that of feeding chickens chopped rabbit meat or viscera, was excluded in two instances but not in the third. It seems possible that wherever wild rabbits have ready access to poultry runs there is some danger of the transmission of the disease from poultry to man. Whenever butchers handle both fowl and rabbits the danger of fowl transmitted disease will be present.

A boy included in this series acquired the disease by handling a quiver made from rabbit skins. The rabbits had been shot and skinned by his father and the skins had been kept in a drawer for about three months before the bow and arrow interest developed. The mother who helped cut and sew the skins also acquired tularemia but was not treated with serum. Primary lesions appeared on the fingers of both mother and son. There were no fly or tick contacts, and no other rabbit or rodent contacts. Each patient was febrile for twenty-nine days, and the boy developed a small area of bronchopneumonia. Hence there was no apparent diminution in virulence of the infecting strain. Cases acquired by handling pelts long removed from animals have been reported from Russia, and Kniazersky and Berdnikov showed by animal inoculation experiments that pelts

⁵ Since this mode of transmission is unusual and possibly new it should be added that a fourth patient, not treated with serum and included in the control group, also contracted the disease from a chicken. This patient, with the typhoidal clinical type, was observed by C. J. Fuoro and F. K. Harder. She was a cardiac invalid and had had no rabbit, fly, or tick contacts. Here also, the source of the chicken was traced to a rural poultry farm that was overrun by wild rabbits.

removed from infected mice and guinea pigs harboured virulent *B tularensis* for forty-five days at room temperatures and for at least six days at 32 to 33°C (18) This is apparently a rare mode of transmission in this country *

Direct man to man transmission continues to be rare despite frequent exposures from dressing primary lesions and incised buboes and from the performance of paracenteses, and in spite of the well established fact that *B tularensis* can penetrate the normal unbroken skin with great rapidity This group furnished an additional example, patients of P A Murr, of Galion, Ohio A woman acquired the infection by penetration of her right hand with a sliver of rabbit bone Her husband had had no contact with this rabbit On the eleventh day of disease he dressed the wound on her hand and fell ill two days later with oculoglandular tularemia⁷ The sources of infection for the patients in the treated group are shown in table 1

Incubation Period

Single exposures permitted determination of incubation periods for 531 patients These are shown in table 2 The only unusual feature is the large proportion of one day periods, including three of less than twelve hours duration

Clinical Types

Table 3 shows the distribution of the 600 cases according to clinical types, also the number of patients with pneumonia and the type pneumonia rates The pneumonia rates for the three bubonic types were greatly exceeded by the pneumonia rate for the typhoidal type Only four patients presented any difficulty in classification according

* Through the courtesy of A D Erehart and T B Rice of Indiana, I have learned of a human case acquired by the bite of a snapping turtle. The source of contamination of the mouth parts of the turtle is conjectural, possibly the viscera or carcass of an infected rabbit left lying on the ground I have not been able to find evidence that turtles or tortoises are susceptible to the disease

⁷ Circumstantial evidence indicates another instance in the case of a physician who performed a thoracentesis on a young man with proven tularemia who had an extensive pneumonia and a large pleural effusion Upon withdrawal of the needle two small drops of pleural fluid fell on one finger and were promptly washed off Two days later the physician had a chill and high fever No primary lesion developed. Pneumonia was present by the fourth day of disease and it rapidly assumed a lobar distribution Death occurred on the tenth day of disease without verification of the etiology of the pneumonia.

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showed that there was always secondary infection with pyogenic cocci, usually streptococci or staphylococci. Good evidences for the existence of an invisible lymphangitis in uncomplicated infections were the scarred, shortened lymph channels that appeared early in

TABLE 2
Incubation periods of 531 patients with solitary exposures

DAYS	NUMBER OF PATIENTS
1	75*
2	110
3	150
4	100
5	44
6	20
7	19
8	3
9	3
10	3
11	2
12	1
13	1
Average	3 30
Mode	3 44

* Includes three of less than 12 hours

TABLE 3
The incidence of the four clinical types and the frequency of pneumonia in the 600 cases

CLINICAL TYPE	NUMBER OF CASES	FREQUENCY	NUMBER OF CASES WITH PNEUMONIA	PNEUMONIA FREQUENCY
		<i>per cent</i>		<i>per cent</i>
Ultero-glandular	525	87.5	80	15.2
Glandular	18	3.0	2	11.1
Oculo-glandular	11	1.8	1	9.1
Typhoidal	46	7.7	24	52.2
Total	600		107	17.8

convalescence. These tough thread like scars always extended from near a primary lesion to the center of a regional lymph node. Many extended from the wrist to the deep axillary nodes and became very prominent if the arm was elevated.

to the method of Francis. All had primary lesions but since none developed adenopathies at any time I could not classify them as ulceroglandular. Except for the primary lesions they had the appearance and clinical courses of patients with the typhoidal type, and they were finally so classified.

Summary of Clinical Observations

In about two thirds of the patients with the ulceroglandular type the primary lesions appeared before attention was drawn to adenopathies, in the other third the buboes appeared first. In the oculo-

TABLE 1
Sources of infection for the 600 acute cases

Rabbits	519
Squirrels	10
Tick bite	6
Cat bite or scratch	5
Chicken	3
Quail	2
Opossum	1
Deer fly bite	1
Pheasant	1
Grouse	1
Skunk	1
Hunting Coat	1
Dressing primary lesion	1
Laboratory work	1
Rabbit skins	1
Unspecified	46

glandular cases conjunctivitis was always present before the regional nodes began to enlarge. Buboes occurred in 554 patients, an incidence of approximately 92 per cent. There were combined ulceroglandular and oculoglandular types of infection in four patients. Nodular lymphangitis was seen in 45 patients, an incidence of 7.5 per cent. The largest number found on one patient was twenty-five, the average was five.

The lymphangitis caused by *B. tularensis* infection alone was never visible to the eye. Whenever inflamed lymphangitic streaks were seen in local cases cultures and smears from the ulcerated lesions

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An exanthem was noted in 120 patients, an incidence of 20 percent. Most consisted of small papular or papulovesicular lesions, and the chief areas involved in order of frequency were back of neck, upper back and shoulders, extensor surfaces of arms and forearms, face, upper part of chest, extensor surfaces of legs, and abdomen. Three patients had very large compound lesions like those of erythema multiforme. No feature of the exanthem is diagnostic of the disease and it is usually regarded as a toxic eruption (19). However, I recovered a pure culture of *B. tularensis* from the heart blood of a guinea pig that had been inoculated with vesicle fluid from one of these patients with a vesiculopustular eruption on the hands. At least some of the skin lesions are therefore true specific exanthemata.

Although not previously recorded for this disease, six patients had the typical lesions of erythema nodosum. They were never solitary, each patient who had them showed from four to twenty lesions. Their appearance, size, development, and evolution were in all ways identical with those which occur in acute rheumatic fever or in tuberculosis. None of these patients had clinical or historical evidences of either tuberculosis or rheumatic fever and it seemed difficult to account for the lesions except on a basis of tularemic etiology. They did not appear during the early, antigenic phase of the infection unless serum treatment had been given during the first week of disease. Most appeared at the end of the second week of disease which marks the beginning of the antibody phase of the infection. These observations support the hypothesis that erythema nodosum is a localized antigen-antibody reaction due to the lodgment of the infecting agent in the skin at a time when homologous antibodies are present in low but increasing concentration.

Multiple primary lesions frequently resulted from the initial contact with infectious material. Primary lesions on the interdigital surfaces spread by contact transfer to the adjacent digits in fifteen patients. One man with a primary lesion on the thumb implanted another one on the shaft of the penis.

In addition to the oculoglandular cases mucosal primary lesions were observed in eleven patients. In five both tonsils were involved, in two others, one tonsil each, also one each on the uvula, posterior pharyngeal wall, base of tongue, anterior faucial pillar, soft palate, and the mucosal edge of one nostril.

Retropharyngeal abscesses developed in two patients, in the third and tenth weeks of disease, respectively. Each one ruptured spontaneously and healed without residual abscess formation. The one observed in the Cincinnati General Hospital contained about an ounce (30.0 cc.) of pus. The rupture occurred during a throat examination to determine the cause of dysphagia. The other one was described to me and was apparently larger. It seems odd that these lymphatic structures were not involved more frequently since tularemia is characteristically a disease of the fixed lymphatic tissues.

Pregnancy was a complication in seven patients. Six women acquired the disease during pregnancies of 3, 4½, 5, 7½, 8, and 8 months duration, respectively. There were no untoward occurrences and all six were delivered of full term, healthy infants. Another woman became pregnant during the third month of tularemia and aborted at the sixth month of gestation, but with no apparent relation to the then completely quiescent infection. The coexistence of pregnancy seemed not to alter the course of the disease or the prognosis. In one instance in which I was able to obtain maternal and cord bloods at delivery the serums from each agglutinated *B. tularensis* in exactly the same dilutions, completely at 1:320 and partially at 1:640.

Albuminuria to slight or moderate degrees was commonly observed during the febrile initial acute phase. Only two patients showed urinary evidences of kidney changes beyond this degree. One, with normal urine at the onset, developed heavy albuminuria without casts or cells early in the third week of illness and died on the thirty-sixth day of disease. The urine boiled solid during the last ten days. The other developed the urinary signs of an acute nephritis during the third week, heavy clouds of albumen, and casts, red and white cells in abundance.

Meningitis occurred in two patients. In each it appeared as a late manifestation of the terminal septicemic phase of the disease. One case has been reported in detail (20).

Encephalitis occurred early in the disease in only one patient who was extremely ill with pneumonia, pleurisy with effusion, pericarditis, femoral thrombophlebitis, and two episodes of acute cardiectasis with extreme tachycardia. The encephalitic symptoms appeared on the eighth day of disease, headache, somnolence, stupor, lateral nystagmus with rapid component to the right, and markedly hypo-

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active superficial and tendon reflexes on the right side. The spinal fluid pressure was greatly increased. Encephalitic symptoms were occasionally noted in other patients but only in the dying state and usually for only a few days before death occurred.

The record of bronchitis is not complete and the incidence of this complication cannot therefore be given. My recollection is that well over one hundred patients seen locally had onsets associated with cough and bronchial rales, but the records reveal only fifty-two instances. Of these, twenty had pulmonary consolidations. Cough with bronchial rales was often present for only a few days at the onset, and it failed to be noticed or recorded when other more striking symptoms overshadowed, and also when patients were first seen subsequent to the second week of disease.

Pleurisy occurred in fifty-six patients, and effusions were present in eighteen. Underlying pulmonary consolidations were demonstrated by physical signs or roentgenograms, or both, in forty-five cases. If the x-ray had been used routinely it is possible that the number of discovered pneumonic areas would have been larger. Three patients with pleural pain, intercostal tenderness, and friction rubs had no demonstrable areas of pneumonia by repeated x-ray examinations.

Pneumonic consolidations were found in 107 patients. Since chest films were not made routinely the observed incidence of pneumonia, 17.8 per cent, is probably a conservative figure although it is in good agreement with the 20 per cent incidence noted by Blackford in his study of 35 unselected cases with repeated chest films (15, 16). Twenty of the 107 patients had extensive bilateral consolidations. Five of these had large pleural effusions that required tapping and one developed three pulmonary abscesses. The remainder had either solitary areas or multiple unilateral areas of consolidation. Although pneumonic areas occurred in any lobe they were noted most frequently in the hilar regions, extending downward into the lower lobes. Since the seasonal incidence of tularemia coincides frequently with a seasonal increase in pneumonia due to other causes there exists a possibility for diagnostic error in regard to the etiology of the pneumonias. Only a few patients were studied carefully to discover other possible infecting agents. Repeated examinations of sputum from six patients

showed a complete absence of Gram positive cocci in five, and a type XIX pneumococcus in one. This patient had a respiratory infection at the time she acquired tularemia. Although still ill she had gotten out of bed to prepare a meal which included wild rabbit. Microscopic examinations of sections of lung removed at necropsy showed organizing lobular pneumonia in some areas and lesions typical of tularemia in other areas. By animal inoculation a pure culture of *B. tularensis* was obtained from the lung at necropsy. If more patients had been studied as carefully as this one it is possible that additional examples of mixed pulmonary infections might have been found. One patient with tularemia and pneumonia suffered an attack of herpes zoster of the thorax over the consolidated area.

Enlarged mediastinal lymph nodes were noted in seven patients with pneumonia and in two patients without pneumonia. They were entirely asymptomatic and were discovered only by x-ray films. Since x-rays were not taken routinely the frequency of occurrence of mediastinal lymphadenitis could not be determined.

Thrombophlebitis occurred in seven patients. The cephalic veins were occluded in two patients, the left femoral veins in four, and both femoral veins in the seventh. This infrequent complication occurred only in the most seriously ill patients, those with pneumonia, pericarditis or pleurisy, or a combination of these lesions. Although it occurred most frequently in the large veins no instance of embolism was noted. Death by embolism has been recorded in the literature only once (21).

Peritonitis with ascites was noted in three patients, and from one of these *B. tularensis* was recovered by direct culture of the ascitic fluid. In one case the development and progress of the abdominal symptoms were similar to those of the case reported by Fulmer and Kilbury (22). In another patient signs of hepatitis, deep jaundice associated with a painful and tender enlargement of the liver, preceded the appearance of the ascites. The third case will be described later in relation to the effects of serum treatment. Hepatitis without ascites was observed in three other patients. Perisplenitis was recognized in two patients by the enlarged, tender, painful spleens and transient audible friction rubs. One of these patients had three severe, brief attacks of splenic pain, on the seventh, fifteenth, and twenty-

fourth days of disease, respectively. A woman with the typhoidal type suffered from a painful swelling in the region of the right ovary. It was not possible to tell whether the mass was the ovary or an enlarged pelvic lymph node. In a man who acquired the disease from a tick bite on the left leg a large mass the size of a small grapefruit, which probably originated in an iliac lymph node, appeared in the left iliac fossa.

Bursitis was seen only once, in an olecranon bursa. On two occasions *B. tularensis* was recovered from the bursa fluid. This case has been reported (17). An unusual feature was the occurrence of transient heart block with complete auricular-ventricular dissociation during convalescence. Two attacks of anginal pain preceded the clinical and electrocardiographic signs of auricular-ventricular dissociation. This man continued to have occasional attacks of anginal pain for many months after recovery from the infection. Death occurred from a coronary occlusion twenty-seven months after his first attack of pain, in the thirty-fifth month from the onset of tularemia.

Complete heart block occurred in another patient, a woman of forty-one, on the thirteenth day of disease. Three days later she developed the typical symptoms and signs of a coronary occlusion which necessitated oxygen administration for three days. Incomplete heart block was seen in another patient, a man of thirty-four. Throughout the prolonged severe initial phase of the disease, complicated by pneumonia, pleurisy with effusion, and mediastinitis, he had an inflexible tachycardia which persisted for one year after recovery. Late in convalescence, two months after discharge from the hospital, he had an attack of severe precordial pain. Electrocardiographic tracings showed partial heart block and signs of coronary disease. Later he was found to have a difference of 30 mm of mercury between the systolic pressures of his right and left brachial arteries which persisted for six months, possibly due to enlargement of the mediastinal lymph nodes.

Pericarditis, with early pain and friction rub followed by an effusion, was noted only once, in a patient who had previously shown the symptoms and signs of a mediastinal pleurisy. Endocarditis due to *B. tularensis* was not observed.

Late recurrent adenopathies appeared in 18 patients, an incidence of slightly more than 3 per cent. In 13 cases they suppurred, an incidence of suppuration of 72 per cent. The stages of disease at which these recurrent nodal swellings occurred are shown in table 4. The frequencies of occurrence and of suppuration did not differ from those observed for untreated cases. Since the pus from liquefied late recurrent adenopathies was always sterile and usually contained foreign body giant cells, indications that these abscesses are usually

TABLE 4
Time of appearance of the late recurrent adenopathies

MONTH OF DISEASE	NUMBER OF PATIENTS	NUMBER WITH SUPPURATION
5th	7	5
6th	3	2
7th	2	2
8th	1	1
9th	2	1
15th	1	0
19th	1	1
25th	1	1
Total	18	13

Due to the shrinking effect of serum administration on the acute buboes it was difficult to distinguish between true late recurrent adenitis as seen in patients in the control group and recurrent enlargements following disappearance of small nodes caused by serum administration to patients in the treated group. The end of the fourth month of disease was arbitrarily selected as the dividing line in order to avoid an excessively high and false incidence of this late complication. There were 46 patients whose buboes had disappeared after serum treatment who experienced reenlargements of these nodes during the second, third, and fourth months of disease. In 35 of these cases suppuration occurred. It is probable that had serum not been given a large proportion of these nodes would have suppurred earlier, nearer the end of the acute initial phase of the disease.

foreign body abscesses and different in mode of origin from the acute abscesses, it was anticipated that serum treatment would have no effect on either their frequency of occurrence or outcome.

EFFECTS OF SERUM TREATMENT

The results of serum treatment varied considerably, especially with relation to the clinical type that was presented, the presence or absence of extensive visceral lesions, the quantity and potency of

serum that was given, and the stage of disease at which patients were treated. The frequent early use of the skin tests permitted verification of diagnoses and administration of serum on or before the twelfth day of disease to 191 patients, almost one third of the total number.

The most constant effect, regardless of the clinical type of disease, was the marked amelioration of the symptoms of intoxication, headache, backache, arthralgias, myalgias, nausea, and mental depression. This effect was usually noted within the first eighteen hours, and most patients obtained marked relief before the seventy-second hour after serum administration. In many cases the transition from a state of utter wretchedness to one of cheerful comfort was accomplished within 24 hours, a response quite similar to those obtainable by the optimal uses of antipneumococcus serums or diphtheria antitoxin.

The most marked changes in the fever curve were noted when patients were treated during the first twelve days of disease, a time when fever rarely disappears from natural causes. Of the 191 patients who were treated before the thirteenth day of disease seven were permanently afebrile in less than 24 hours, thirty-two became permanently afebrile before the seventy-second hour, sixty (31 per cent) were afebrile before the end of a week, and ninety-seven (51 per cent) were fever-free before the end of the thirteenth day after serum administration. As noted previously, all days of fever due to serum sickness were included. The average day of disease on which serum was administered was the eighth day, and the average duration of fever thereafter was 6.1 days.

The shortest recovery periods were observed in patients who had the typhoidal type of disease without pneumonia. There were 21 such patients. The average day of disease on which serum was given was the thirty-fifth. The average duration of disability was two months, and the average period between administration of serum and resumption of full activities was 25 days. The effect of serum administration on the fever curve of one of these patients who was treated on the eighth day of disease is shown in figure 5.

The importance of pneumonia as a factor which prolonged disability and retarded recovery is well shown by the results of treatment in 18 patients with the typhoidal type with associated pneumonias. There were no lymphadenopathies to contribute to the prolongation

of disability in these cases. The average day of disease on which serum was administered was the same as it was for the previous group, the thirty-fifth day, but the average duration of disability was 3.5 months and the average recovery interval was 66 days, more than twice as long as it was for the group of typhoidal type patients without pneumonia. The effects of early serum treatment in typical cases of the typhoidal type with pneumonia are shown in figures 6 and 7.

A characteristic and almost constant feature of the therapeutic response in patients with the bubonic forms, the oculoglandular,

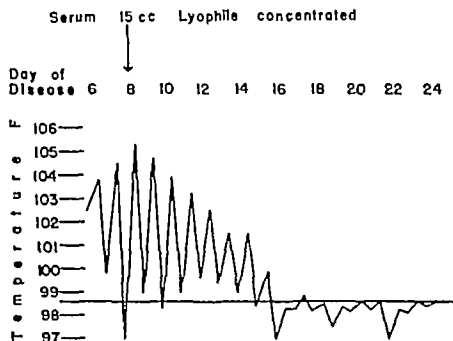


FIG 5 Female 26, a cook, infected by washing a purchased wild rabbit. Typhoidal clinical type without physical or roentgenologic evidences of pneumonia. The spleen was readily palpated on the eighth day of disease when the equivalent of 300 cc. of anti tularemic horse serum was administered intravenously in one dose of 150 cc. The patient was without complaints 72 hours after serum administration. There were no complications and no serum sickness. The spleen could not be palpated after the twentieth day of disease.

glandular, and ulceroglandular clinical types, was a prompt and marked reduction in the sizes of the buboes and amelioration of the pain caused by them. Following the initial diminution the nodes frequently fluctuated considerably in size before they either dwindled toward ultimate disappearance or enlarged to become abscesses. Buboes that had already reached or exceeded a diameter of five centimeters at the time serum was given frequently failed to show this shrinkage. Furthermore buboes of this size usually suppurred, and it was noted that additional serum over the amount indicated by the general status of the patient did not prevent either the immediately

impending suppuration or the later liquefactions of these large nodes. When primary lesions were present serum administration resulted in a lessening or disappearance of the localized pain and accelerated healing.

With allowance for variability in the presence and in the location of primary lesions, the clinical appearance, course of disease, and frequency of pneumonia were very similar in patients with the oculo-glandular, glandular, and ulceroglandular types of disease. The systemic and regional effects of serum treatment were also very similar.

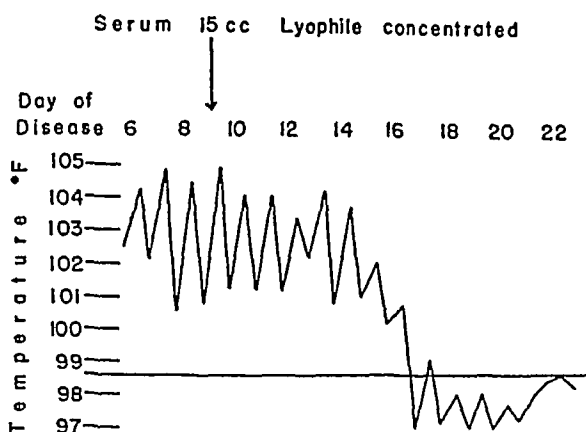


FIG 6 Male, 18, poultry cleaner, infected by skinning wild rabbits. Typhoidal clinical type with sore throat at onset, umbilical pain, nausea and vomiting. Had a scattered papulopustular exanthem of face, chest and back. No symptoms or signs of pneumonia during the first six days. A left hilar pneumonia was demonstrated roentgenologically on the seventh day of disease, associated with cough and bronchial rales in the left lung. The spleen became palpable on the eighth day and a pleural friction rub was noted in the left axilla on the ninth day at which time 15 cc of double concentrated lyophile horse serum were administered in one intravenous injection. Although fever persisted for four additional days the patient was otherwise entirely symptom free 72 hours after serum administration.

Figure 8 shows the response to treatment in a child who had the oculo-glandular type of infection. The fall in temperature, accelerated healing of the primary lesions, and the initial shrinkage, subsequent swelling, and fairly prompt disappearance of enlarged lymph nodes are typical of the results obtained in children. Figure 9 shows the effects of treatment on this type of the disease in an adult.

The effect of one small injection of serum on the fever curve and on an accessible enlarged node of a patient with the glandular type of infection is shown in figure 10. The clinical courses of the 18 pa-

tients with the glandular type and the 525 patients with the ulceroglandular type were quite similar, and all measurable aspects of the disease in these types fell within the same limits of variability

Although the presence of pneumonia and other regional localizations of infection were important causes of serious and prolonged disease in patients with the ulceroglandular type the most common

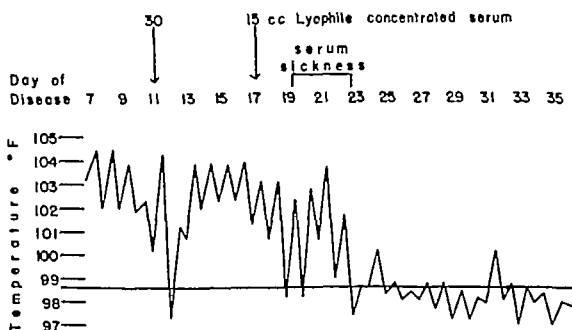
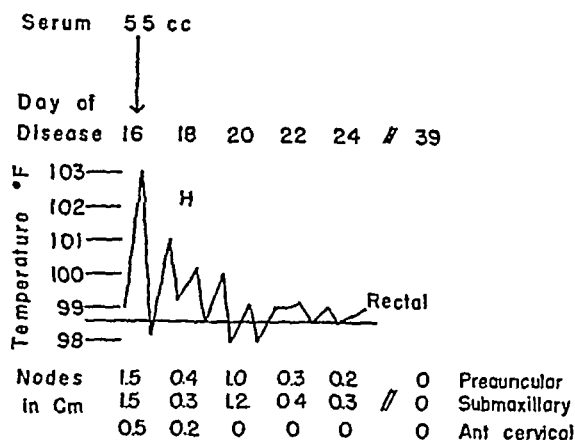


FIG 7 Male, 42, typhoidal type, with extensive pneumonia involving most of the left lung and a large left pleural effusion. Immune serum tests positive on the 7th and 11th days of disease. Bacterial skin test positive on the 11th day of disease. Agglutination test negative on the 7th day of disease, positive to 1:20 on the 11th day, to 1:80 on the 14th day and to 1:320 on the 18th day of disease. *B. tularensis* stained in and recovered from 950 cc. of pleural fluid removed on the 15th day of disease. Lyophile horse serum, restored to half volume, administered on the 11th and 17th days of disease. There was roentgenologic evidence of resolution of the pneumonia by the 24th day of disease. The remainder of the pleural effusion was absorbed before leaving the hospital. There was serum sickness of moderate severity which lasted for 96 hours. This patient's nephew infected on the same rabbit hunt, was hospitalized in an adjacent bed, with the ulceroglandular clinical type, pneumonia, and pleurisy with effusion. The clinical course and response to serum therapy were almost identical with those of the uncle. A female neighbor to whom these men gave one of their recently shot rabbits acquired a severe febrile, prostrating disease two days later, developed an extensive pneumonia four days later, went to another hospital where she died on the tenth day of disease with a diagnosis of pneumonia of undetermined etiology.

causes of protracted disability were the persistence of painful buboes, their frequently delayed suppuration, and the draining and dressing of the wounds after the abscesses had been incised. The patients that were treated before the thirteenth day of disease had suppurative adenitis less frequently than those who were treated at later stages. However, the earliest possible treatment did not prevent all nodal suppuration. The lowest incidence of suppuration that was obtained



H = Primary lesion healed

FIG 8 Boy, 5, infected by playing with a knife that had been used to decapitate a recently shot rabbit. Oculoglandular clinical type involving the right eye and regional lymph nodes. No pneumonia. Papulopustular exanthem on face and arms. The conjunctivitis was in a subsiding stage when 5.5 cc of goat serum were given intravenously on the 16th day of disease. The conjunctivae were normal in appearance and the exanthem had almost completely disappeared 48 hours later. A slight degree of enophthalmos of the right eye persisted for 15 months.

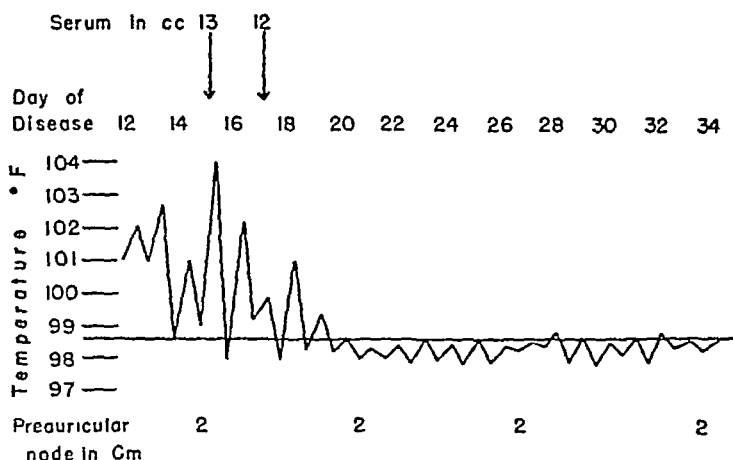


FIG 9 Female, 27, housewife, infected by preparing a rabbit. Oculoglandular clinical type involving the right eye and the regional lymph nodes. No pneumonia. Goat serum was administered intravenously on the 15th and 17th days of disease. The condition of the eye was normal on the 30th day of disease. The enlarged preauricular node remained undiminished in size for several months. It is not known if suppuration occurred eventually. Repeated serum agglutination tests resulted in the highest titers I have seen recorded. On the twelfth day of disease the titer was 1:20. On the sixteenth and eighteenth days of disease, after serum administration, the titers were 1:320. From the twenty-first to the forty-ninth days of disease four additional tests were performed at intervals of approximately one week. These tests showed complete agglutination of B tularensis in dilutions of 1:2560, 1:5120, 1:40,280 and 1:80,560, respectively. This case record was made available through the courtesy of Dr. Bernard Weinstein of the Vanderbilt University Hospital.

under optimal conditions of early diagnosis, and with the total recommended amount of serum given before fluctuation could be detected, was 33 per cent. This is somewhat more than half the frequency of suppurative adenitis that occurs without serum treatment. The effect of treatment in preventing suppuration of the dermal lymphangitic nodules was more apparent. Only ten of the forty-five patients with nodular lymphangitis suffered liquefactions of one or more nodules, an incidence of suppuration of 22 per cent, approximately one third of that for the control group. Examples of the failure of early treatment to prevent the rapid suppurations of buboes are shown in figures 11 and 12. The effect of treatment in delaying

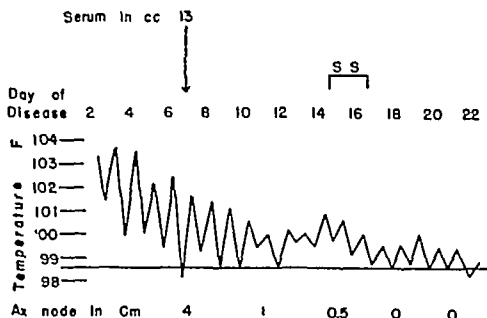


FIG 10 Male, 38, infected from wild rabbits. Glandular clinical type, axillary bubo but no primary lesion. One small intramuscular injection of goat serum was given on the seventeenth day of disease. This was followed by a gradual decline in fever interrupted for two days by mild serum sickness, and a fairly prompt disappearance of the bubo.

suppuration and thus prolonging disability is shown in figure 13. This chart also illustrates the poorest effect of serum administration on the temperature curve that was seen and an example of severe and rather prolonged serum sickness. In other cases treated during the first ten days of disease there occurred a prompt and permanent disappearance of the buboes. Figure 14 shows a notable shortening of the febrile period and the rapid disappearance of a small bubo. In this case serum was administered during the period of primary febrile remission. Figure 15 shows a very marked alteration of the fever curve which followed serum administration at the height of the secondary rise in temperature, later complicated by severe serum

sickness The unusual features of this case were the extremely large axillary bubo, its very rapid reduction in size during the four days after serum administration, its continued and steady regression after serum sickness had passed, and the fact that it did not suppurate during the year of subsequent observation

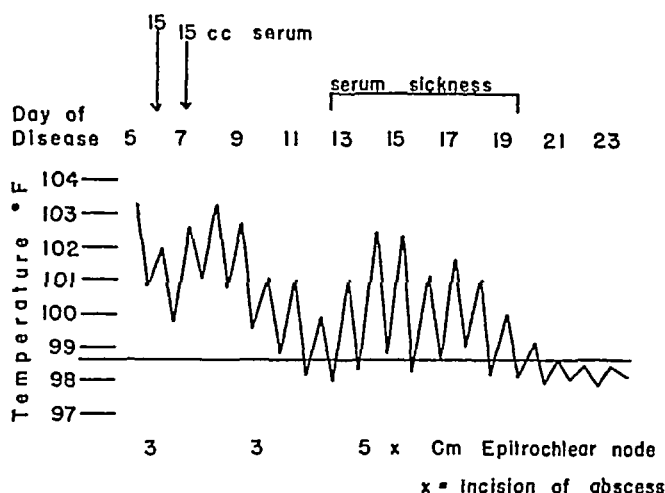


FIG 11 Male, 34, butcher Infected by cleaning wild rabbits Ulceroglandular clinical type Unilateral primary lesion on right third digit with bilateral axillary adenitis, unilateral epitrochlear bubo, and nodular lymphangitis on the right forearm Goat serum administered on the sixth and seventh days of disease exerted a good effect on the temperature curve until moderately severe serum sickness supervened Early therapy did not alter the rapidly progressive suppurative course of the bubo at the elbow The mass enlarged rapidly with the onset of serum sickness, a very frequent occurrence. The abscess was incised on the sixteenth day of disease and the incision was completely healed by the twenty-eighth day The lymphangitic nodules and the axillary nodes did not suppurate The superiority of the skin tests over the agglutination test for early confirmation of the diagnosis was well demonstrated in this case The bacterial suspension and the antiserum skin tests were both positive on the sixth day of disease Agglutination tests were negative on the sixth, twelfth, fourteenth, and sixteenth days, and positive to 1:160 titer on the nineteenth day of disease, thirteen days after serum had been administered

Some of the first patients to be treated with serum had pneumonia, and most of these were considered to have made very satisfactory recoveries following the administration of the usual 30.0 cc doses of serum As experience with the treatment of pneumonic patients increased it became apparent that this dosage was frequently too small In the more seriously ill individuals the improvement that followed the initial administration was not lasting In a few days the symptoms of intoxication returned, the temperature rose again or

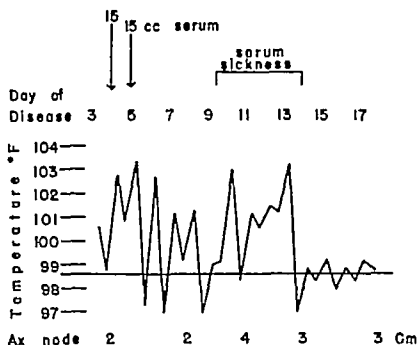


FIG 12 Male 19, butcher Ulceroglandular clinical type with unilateral primary lesion and bilateral axillary adenitis. There was marked alteration of the temperature curve after the intravenous administration of goat serum on the fourth and fifth days of disease, later complicated by serum sickness. The primary lesion was healed by the tenth day of disease. Bilateral axillary buboes of equal size did not become smaller after serum was given. Each enlarged with the advent of serum sickness and then receded gradually but always remained larger than the sizes first noted. Both enlarged again during the fourth week, suppurated, and were incised during the fifth week.

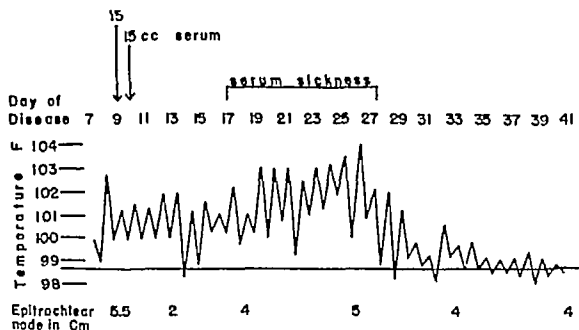


FIG 13 Female 64 housewife Infected by market rabbits Ulceroglandular clinical type with right axillary and epitrochlear adenitis. Horse serum was administered intravenously on the ninth and tenth days of disease. This chart illustrates the least effect of serum treatment on the temperature curve that was noted in the entire series. The febrile course was complicated by prolonged and severe serum sickness. The epitrochlear bubo diminished to less than half its previous diameter after serum was given. It re-enlarged with the onset of serum sickness and fluctuated in size thereafter for another month. Suppuration finally occurred and the abscess was incised on the 66th day of disease. The axillary node re-enlarged and also suppurated a month later. Had serum not been given it is probable that both nodes would have suppurated sooner than they did.

remained unaltered, the consolidated areas increased in size, and in some cases new areas of pulmonary or pleural infection appeared. An example of this kind of response to inadequate early dosage is shown in figure 16. In marked contrast is the temperature curve in figure 17 which shows the effect of two consecutive daily injections of concentrated restored lyophile serum in a severely intoxicated patient with extensive intrathoracic lesions. Before a minimal initial dosage of 60.0 cc of serum was adopted as routine treatment for all patients with pneumonia five deaths had occurred, one in Cincinnati

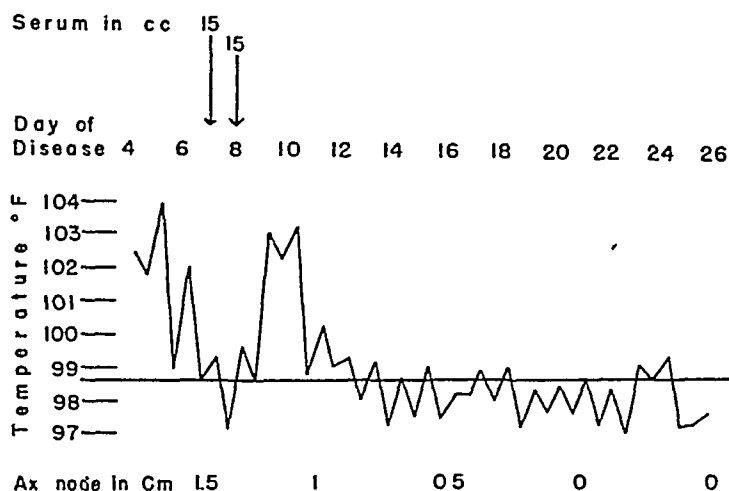


FIG 14 Male, 43, infected by cleaning wild rabbits. Ulceroglandular clinical type with bilateral primary lesions and bilateral axillary and epitrochlear adenopathies. Papular exanthem on neck, chest and upper back. Goat serum was administered intravenously during the primary febrile remission on the seventh day of disease. The marked alteration in the secondary febrile period is shown, also the rapid disappearance of the largest of the accessible lymph nodes. Serum sickness did not occur and no nodes re-enlarged or suppurated. Both skin tests were positive on the fourth day of disease. Agglutinins were still absent on the eleventh, eighteenth and twenty-first days of disease.

and four elsewhere. In each case transient improvement followed the injection of smaller amounts of serum. Since there were no symptoms or signs of septicemia in any of these patients at the time serum was given it seems probable, in retrospect, that had larger amounts been given most of these deaths might have been prevented. Eventually it was noted that whenever the initial 30.0 cc dose of serum given to a patient without apparent pneumonia did not result in a continued lowering of temperature and a marked symptomatic improvement by the seventy-second hour a chest film usually revealed an area of pneu-

monia that had not been detected by physical signs, and that if an additional 300 cc of serum was then given at once it usually sufficed to induce a good recovery

A report has been made of the favorable results of treatment of a patient who acquired the disease from a tick bite and who had had pneumonia and pleurisy for several weeks before serum was given. In this case an organism similar to *B. tularensis* had been recovered

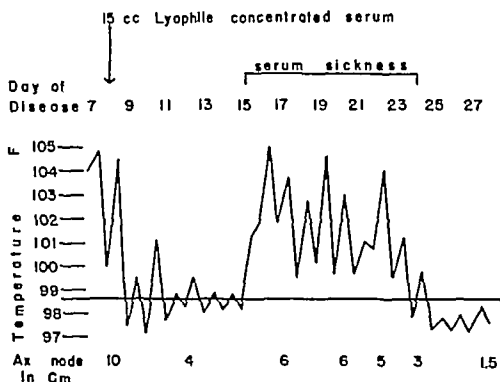


FIG 15 Male, 28 poultry cleaner. Ulceroglandular clinical type with unilateral primary lesion and bilateral adenopathies. Severe initial phase without cough and without physical or roentgenologic signs of pneumonia. Concentrated restored lyophile horse serum was administered intravenously at the height of the secondary rise in temperature on the eighth day of disease. From the eleventh until the fifteenth days of disease, when serum sickness occurred the patient was completely asymptomatic. Although lymph nodes that exceed a diameter of 5 centimeters almost always undergo eventual suppuration the large axillary bubo in this case proved a rare exception. Although serum sickness continued for eight days, accompanied by high fever, there was little associated distress since arthralgias did not occur and urticaria appeared for only two periods of four hours each

by blood culture (23). Another patient, with bilateral basilar consolidations, had fever ranging from 104° to 106°F for eight days prior to administration of 600 cc. of serum on the fourteenth day of disease. There was also thrombophlebitis of both femoral veins. This patient was afebrile and without signs of venous inflammation or obstruction on the twenty-eighth day of disease.

The effect of treatment given late in the course of disease to a patient with progressive pulmonary lesions and persisting infection in

the serous cavities is illustrated by the following case. A woman acquired the ulceroglandular type of disease in November, soon followed by signs and symptoms of pneumonia which showed steady progression. By February there was a large pleural effusion on the right side and a peritonitis associated with ascites. Abdominal paracentesis yielded three liters of fluid from which *B. tularensis* was

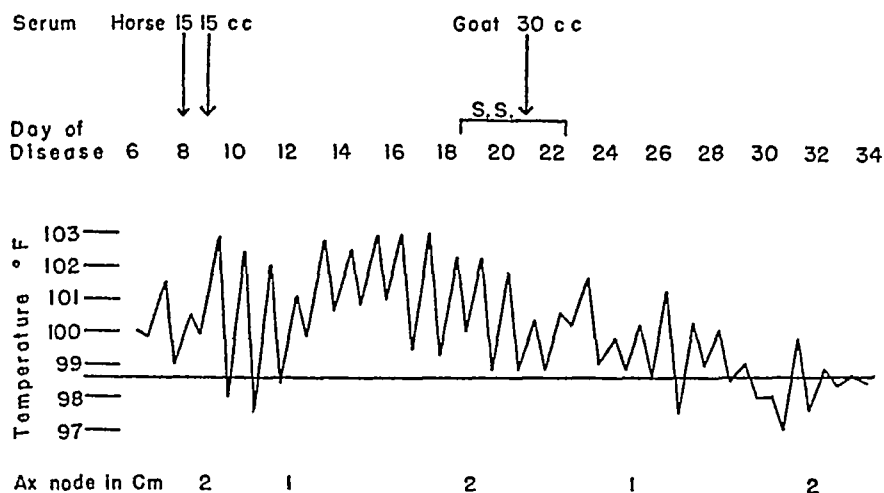


FIG 16 Male, 39, ulceroglandular type. Onset with vomiting, abdominal pain, and toxic ileus with extreme abdominal distension, tympanites, and profound intoxication. A small area of pneumonia in the right hilus, discovered on the thirteenth day of disease by physical signs and chest films, continued to enlarge after the first divided doses of serum. By the nineteenth day of disease the pneumonic area occupied most of the right lower lobe and there was a painful dry pleurisy in this region. On the next day the pleurisy extended to the mediastinum with excruciating substernal and epigastric pain. There had been no change in the abdominal condition and the patient became confused and delirious, with the general condition aggravated by the supervention of serum sickness. The indication for more serum was urgent. Goat serum was given to avoid the likelihood of anaphylaxis consequent upon reintroduction of horse serum during the stage of serum sickness. This induced a marked general improvement. The patient was mentally clear in 24 hours. The abdomen was relaxed and soft in three days and the pleuritic pain disappeared the following day. Recovery ensued and there was x-ray evidence of resolution of the pneumonia before discharge from the hospital. The enlarged lymph node continued to fluctuate in size during the next three months. It finally suppurated and was incised on the 124th day of disease.

recovered by culture. The peritonitis subsided slowly but the thoracic signs indicated persisting and extending pleuropulmonary involvement. Fever was continuous. In April the pleural effusion reached large proportions and three thoracenteses were performed. The general condition persisted essentially unchanged. Early in May, on the one hundred seventy-ninth day of continuously febrile

and bed ridden disease, 300 cc. of serum were administered. This was followed shortly by marked symptomatic improvement, subsidence of fever, resorption of pleural exudate, and slow resolution of the chronic pneumonitis. Fever disappeared permanently on the sixteenth day after serum administration. Recovery was practically complete when the patient left the hospital eleven days later. The previously cited patient with encephalitis, pneumonia, pleural effusion, pericarditis, thrombophlebitis of the left leg, and acute cardiac

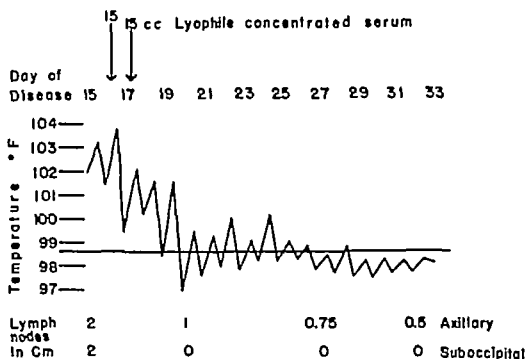


FIG. 17. Male, 33, ulceroglandular type, with pneumonia involving most of the right upper lobe and a dry pleurisy over the left lower lobe. Symptoms of pneumonia existed from the day of onset. Lyophile horse serum, restored to half volume, was administered on the 16th and 17th days of disease, followed by a rapid lysis of temperature and shrinkage of buboes. There was x ray evidence of resolution of the pneumonia on the 20th day of disease. The enlarged suboccipital node had returned to normal size and was barely palpable on the fourth day after the first serum injection. The right axillary node diminished steadily in size. There was no subsequent enlargement or suppuration. The advisability of prompt initial administration of not less than 60 cc. of serum to patients with pneumonia is illustrated by comparing this chart with the chart in figure 16. Similar contrasts were observed between six other pairs of patients who were treated as these were.

failure was given 600 cc. of serum during the seventh week of illness. Within five days the progressively downward course had changed to one of steady improvement, and this man was back at his work three and a half months later with no trace of residual disability.

Deep jaundice with moderate enlargement of the liver but without signs of ascites, pneumonia, pleurisy, or other deep tissue involvement had developed slowly from the second week of illness in another patient. He had lost thirty pounds (13.6 kg) in weight by the six-

tieth day of disease when 300 cc of serum were administered. A restoration to normal color, weight, and strength followed with amazing rapidity, and the patient went back to work apparently entirely well ten days after serum had been given. Although a diagnosis of hepatitis had been made the rapidity of complete recovery suggested strongly that the chief lesion had been lymphadenitis of the hepatic hilar nodes with gradual compression and obstruction of the bile ducts and that the shrinkage of enlarged nodes which usually follows serum administration had released the pressure on the ducts with consequent restoration of the normal flow of bile.

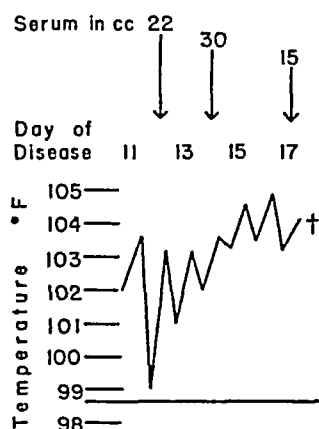


FIG 18 Male, 67, infected by cleaning wild rabbits. Ulceroglandular clinical type with unilateral primary lesion and axillary adenitis. Onset and early course fairly mild. On the tenth day of disease a chill was followed by high fever which remained sustained. He became rapidly confused, delirious, stuporous and then comatose. The liver and spleen enlarged rapidly and slight icterus appeared. Multiple areas of pneumonia developed in each lower lobe. The first intravenous injection of goat serum resulted in a prompt fall in temperature from 103 to 99°F and a return of mental clarity. After four hours the fever and delirium recurred and remained unaffected by additional injection of serum. The first serum injection was given about 48 hours after the onset of septicemia.

The urine of the patient with hemorrhagic nephritis became normal on the seventh day after administration of serum and the ensuing recovery was uneventful.

The ten patients who were given serum after the symptoms and signs of septicemia had been present for from two to five days did not survive. Dosage of serum ranged from 66 to 360 cc per patient. Following the initial injections there was usually a brief lowering of fever accompanied by a transient period of mental clarity but this effect seldom persisted for more than six hours. The temperature

curve in figure 18 is typically representative of this small group of patients who were first seen in the dying state. The patients who received larger amounts of serum showed no better response. Blood cultures from two of these patients yielded colony counts of approximately 3000 and 8000 colonies of *B. tularensis* per cubic centimeter of blood, respectively. Figure 19 shows the temperature curve of the patient who acquired tularemia before she had recovered from a pneumococcus bronchopneumonia.

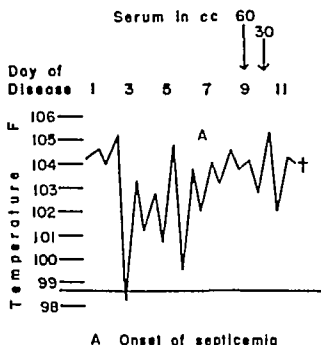


FIG 19 Female, 48, infected by preparing a market rabbit. Ulceroglandular clinical type with unilateral primary lesion and adenopathies. An asthmatic with arterial hypertension massive cardiac hypertrophy, and previous attacks of angina and periods of myocardial failure. Had not recovered from an acute respiratory infection before she acquired tularemia. *Pneumococcus* Type XIX was recovered from sputum. Confusion, delirium, and a change in the character of the fever curve from remittent to sustained occurred on the seventh day of disease and probably marked the onset of tularemic septicemia. Goat serum was not administered until 48 hours later and it exerted no detectable beneficial effect. A pure culture of *B. tularensis* was recovered by guinea pig inoculation with pulmonary exudate obtained at necropsy.

In only one instance was it possible to start treatment of a patient with septicemia within the first few hours from the onset of this heretofore invariably fatal complication. The unexpected and dramatic recovery which followed was attributed solely to the promptness with which large amounts of serum were administered after the appearance of the symptoms and signs of onset of septicemia. The patient, a surgeon with arterial hypertension, had acquired the infection from quail. His temperature curve is shown in figure 20. Although he had never had them previously, he began to suffer from

attacks of angina pectoris during the third month from onset, and these attacks have continued ever since. At the time of writing, four and one half years after onset of illness, this patient still lives and, to my knowledge, is the only person ever to have recovered from tularemic septicemia.

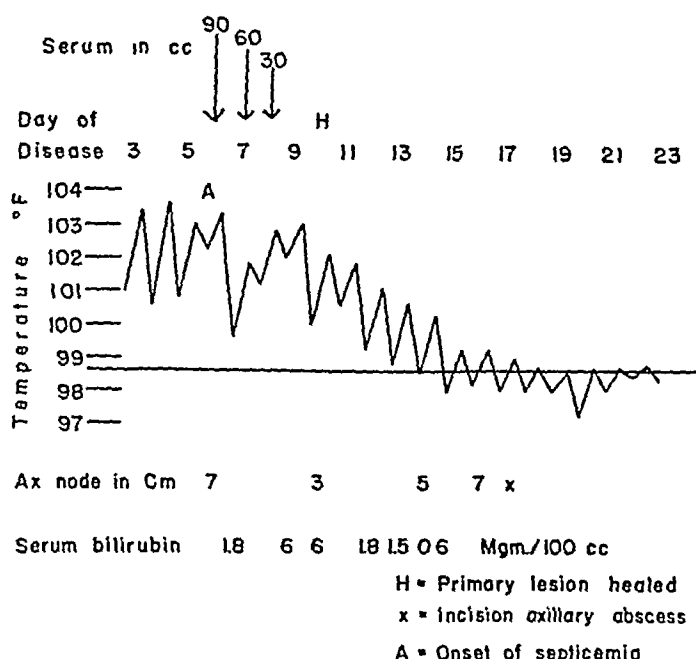


FIG 20 Male, 60, physician. Infected while hunting quail. Ulceroglandular clinical type. Severe illness from onset, with scattered areas of bronchopneumonia, hepatitis, increasing jaundice, profound intoxication and massive enlargement of the right axillary nodes. Fever was septic in character until the morning of the sixth day of disease when it became non remittent. There was associated delirium, confusion, and stupor, and he became semicomatose before noon, with deepening jaundice and enlargement of the liver and spleen to 8 cm. below the costal margin. First horse serum administration was at 5 15 P M. He was mentally clear the next morning and he remained so thereafter. A total of 180 0 cc of serum was administered during a period of 41 hours. Although a culture had shown that the primary lesion on the little finger was secondarily infected with hemolytic streptococci the lesion healed very rapidly. There was a prompt reduction in the size of the axillary node, soon followed by reenlargement, suppuration, and incision. The incision drained for 50 days. Bacterial suspension skin test positive on the third day of disease. Serum agglutination tests negative on the third, seventh, ninth, and tenth days of disease.

The charts and protocols indicate that horse serum and goat serum were used interchangeably in the same dosage. When alternate series of patients were treated with each serum there were no detectable differences in apparent potencies as judged by clinical effects. Clin-

ical evidence indicated that better results were obtained when patients were treated before the thirteenth day of disease. Although this is believed to be the optimal time to use serum it should be emphasized that experience with 144 patients indicated that this form of treatment was very effective in accelerating recovery and in terminating continuous or intermittent disease from the second to the ninth month after onset. On the other hand there is apparently a dead line somewhere between the tenth and fourteenth months of disease beyond which serum therapy rapidly becomes totally ineffective. This was indicated by the results of trial therapy in twelve patients who had been continuously ill for periods ranging from fourteen months to five and one half years at the time serum was administered. Only one patient recovered, the one treated in the fourteenth month of disease. The other eleven became afebrile and asymptomatic in from two days to two weeks but this effect lasted at the most for only a few weeks, in some for only a few days. Relapses occurred promptly, and each of these patients is still ill with continuous or intermittent symptoms of persisting tularemic infection. Five of these patients were inoculated carefully with tolerance doses of a specially prepared *B. tularensis* vaccine, daily, for many months. Although it had been shown previously that this vaccine was therapeutically effective for 16 patients in the earlier stages of the infection it failed to cause any improvement in these patients in the late chronic stages. Neither serum nor vaccine appeared to be of any value after the disease had persisted for a year or more.

Effect of Treatment on Complications and Sequelae

One patient suffered a single relapse or recrudescence. Near the end of the third month of severe illness, the typhoidal clinical type with pneumonia, this woman had been given 300 cc. of horse serum and had made a very slow recovery. The relapse occurred twelve months after serum administration, and she was then given 300 cc. of goat serum. Convalescence was slow and not complete for five and one half months thereafter. No further relapses occurred and she has remained well during the subsequent forty four months of observation. Although a number of reports record relapses and recrudescences (17) there is no accurate information concerning their

frequency of occurrence It may be noted that the patient that suffered the relapse was not given as much serum as would now be recommended for a patient with pneumonia

No surviving patient of the treated group developed the chronic form of the disease Although the frequency of occurrence of chronic tularemia is unknown the number of examples of this phase of the infection that have been encountered, fifteen during the six years of this study, suggests that serum therapy may help to prevent the extension of the disease into the chronic stages This supposition gains some additional support from the observed results of treatment given late in the course of the disease The administration of serum effected complete recoveries in 24 patients who had been continuously or intermittently ill three to eight and one half months from the onsets of disease These patients were observed for a minimum of three years after recovery occurred

Tularemic septicemia, the most serious complication and the most frequent cause of death, did not occur after serum administration in any patient who had received the optimal amount of serum

The patient that developed the urinary signs of an acute nephritis showed a marked diminution in the quantities of albumen, casts and red blood cells five days after serum administration The urine was normal two weeks later

No persisting extensive fibrotic pulmonary changes were noted Pulmonary abscesses healed completely without residual detectable changes Bronchiectasis did not occur

Of the six surviving patients with thrombophlebitis one had edema of the leg for five months, the others showed no sequelae longer than three weeks after treatment had been given

None of the patients with hepatitis or peritonitis has so far shown any gross evidences of residual liver damage or recurrence of ascites

Fibrosis of invaded subcutaneous lymph channels occurred frequently The end results were tough, shortened, thread-like cords that extended from near a primary lesion to the center of a regional lymph node When they stretched across the axilla they frequently caused a restriction to full elevation of the arm, and local pain With graduated elevation exercises the disability rarely lasted for more than three weeks In some cases the local pain was referred to the shoulder

joint, and if the cords were not seen at first an erroneous diagnosis of arthritis was usually made

Treatment of Special Symptoms

Buboes For relief of pain the most satisfactory local treatment was a warmed wet dressing of saturated aqueous solution of magnesium sulphate. When liquefaction occurred it was found best to delay incision until the node capsule had perforated, forming a definite pouching soft area that was easily palpated. The most frequent result of premature incision was a very prolonged period of drainage, with delayed healing of the incision and occasional residual abscess formation. Secondary infection occurred frequently when nodes were incised too early. When the abscess is ripe simple incision and drainage is all that is necessary. Excision of nodes is not recommended. It is not always successful in removing all infected nodes, it does not prevent enlargement and abscess formation of adjacent uninvolved nodes, it is more surgery than the condition warrants, and it sometimes results in distressing and prolonged lymphedema.

Eye Lesions The use of dyes, silver preparations, and special solutions has not been notably beneficial (24). A satisfactory treatment consists of continuous hot applications of half saturated aqueous magnesium sulphate and frequent lavage of the conjunctival sac with warmed boric saline solution.

Dermal Primary Lesions The best local treatment was found to be a wet dressing of one half to one third saturated aqueous solution of commercial urea. Unless the dressing caused pain the stronger solution was preferable. Not one of the many lesions treated with urea became secondarily infected. The next best treatment was saturated solution of magnesium sulphate but about one in twenty that were treated in this manner became secondarily infected, usually with staphylococci. Despite much published advice to the contrary many physicians continue to incise the primary lesions, apparently in the belief that they are dealing with a pyogenic infection. This procedure is not only useless but often demonstrably harmful. Thirty two patients in this series suffered sharp attacks of chills and fever within a few hours after their primary lesions had been incised.

Importance of Previous Heart Disease Heart disease was an im-

portant cause of prolonged disability and of mortality. Fourteen surviving patients were known to have had heart disease before they acquired tularemia. Those with hypertensive heart disease remained notably free from cardiac complications whereas every patient with rheumatic or vascular disease suffered some degree of muscle failure, usually after the fourth week of tularemia. A brief summary of these cases is presented in table 5. Eight surviving patients who were not suspected of having heart disease suffered cardiac complications, usually during convalescence from the infection. Seven had coronary occlusions with anginal pain and acute myocardial failure, and the eighth patient probably had a painless coronary occlusion. These cases are summarized briefly in table 6.

Careful questioning of each patient failed to elicit any symptoms of heart disease prior to the onset of tularemia.

The accumulated necropsy experience does not permit the assumption that these eight patients had normal hearts at the time tularemia was acquired, and that this infection produced new vascular lesions in the cardiac circulation. Although obliterating arteriolar lesions due to intimal proliferation have been seen occasionally in other organs they have been conspicuously absent in the heart. Cloudy swelling, loss of striations, fragmentation of muscle fibres, and sparsely scattered focal cellular infiltrations between muscle bundles have been the only myocardial lesions noted. These lesions have seldom been severe or extensive, and they have not occurred frequently (13). There is little or no existing evidence that tularemia seriously damages the normal heart. The greater probability is that this severely intoxicating infection causes latent coronary disease to become manifest. With this in mind I have been predicting for several years that whenever death occurred to any patient who did not have tularemic septicemia and who had had the optimal amount of serum a serious cardiac lesion, probably of vascular origin, must have been present. This brash prophecy was intended only as a stimulus to get an increased number of necropsies or to search back records for previous evidences of cardiac disease in such patients, but whenever these tests have been applied there has been no failure to date to find valid evidence of previous heart disease.

TABLE 5

Patients with known pre-existing heart disease

PATIENT	SEX	AGE	NATURE OF HEART DISEASE	CLINICAL TYPE	DAY OF DISEASE SERUM GIVEN	REMARKS
W J R.	M	53	Old rheumatic heart, mitral stenosis	T	22	Recovery; disability prolonged 3 months due to heart condition
E D	F	52	Rheumatic carditis, mitral valvular dis.	U	15	Usual course and recovery, acute heart failure 4 months after onset, slow recovery, still lives
L. B	F	55	"Chr myocardial disease" of 1 yr	U	29	Convalescence prolonged about 3 months
D H	F	50	Hypertensive heart disease	U	3	No delay in convalescence, rapid complete recovery
M C.	F	55	Thyroid adenoma, tachycardia, hypertension	U	6	Short convalescence, no cardiac symptoms
J U	M	56	Arteriosclerosis peripheral and cardiac	U	2	2 months after onset had acute dyspnea and substernal pain, short of breath 2 weeks
F H	F	29	Essential hypertension	U	59	No tularemia complications but convalescence prolonged to 9.5 months, an excess of about 7.5 months, due to heart failure
R F	M	65	"Myocardial disease"	U	22	No tularemia complications but convalescence prolonged to 12 months due to heart failure
A. K.	F	65	Hypertensive heart disease	U	62	Prompt and uneventful convalescence
M F	F	65	Hypertensive heart disease	U	11	Prompt and uneventful convalescence, disabled for only 12 days
G B	M	53	Hypertensive heart disease	U	6	Normal convalescence, total disability 16 months
L C.	F	64	Hypertensive heart disease	U	9	Uneventful, but prolonged convalescence, an excess of about 10 month
A. Th	F	46	Hypertension, obesity, mild cardiac failure	U	4	Normal course total disability 27 days
B R.	F	24	Patent ductus arteriosus	U	23	Normal course and convalescence

TABLE 6
Patients without evidence of previous heart disease

PATIENT	SEX	AGE	CLINICAL TYPE	DAY OF DISEASE SERUM GIVEN	REMARKS
A G	M	44	U	5	Several attacks of precordial and epigastric pain 1 mo after serum, lasting several weeks None for 4 years
C H	F	41	U	4	Usual convalescence Total disability 17 mo Early in third month abrupt onset of orthopnea, tachycardia, dependent edema, vertigo No pain Lasted for 6 mo, then incomplete recovery Mild heart failure for next 2 years Still lives
J T S	M	43	T	255	"Cardiac" pain, probably pleural, in first week of onset. Precordial pain, chills, slight fever, weakness, in sixth month of disease, also 1 month later EKG showed myocardial disease Infrequent, but continuing, attacks of angina for next 3 years All residual symptoms and signs of tularemia disappeared 12 days after serum
E W	F	54	U	2	Normal course and early convalescence Felt well at third month Early in fourth month severe angina attack with severe heart failure that lasted 5 months No subsequent pain Slight heart failure persists for 19 months
B S	M	60	U	6	Recovered from tularemic septi-cemia Onset of severe attacks of anginal pain in third month from onset. These have continued intermittently for 3½ years
W H	M	49	U	16	Prompt recovery from acute phase of tularemia Coronary occlusion and heart block in eighth month from onset Final occlusion, and death, in thirty-fifth month from onset
R G	M	34	T	20	Coronary occlusion and partial heart block in fourth month of disease
F S	F	41	O	13	Coronary occlusion on sixteenth day of disease Moderate muscle failure persists

Six deaths appeared to be caused primarily or entirely by heart disease

Case 41 Male, 63, typhoidal type Was recovering slowly from the severe initial phase when 25 cc. of goat serum were given intravenously on the 41st day of disease The onset had been severe, with marked distension and deep abdominal pain He became afebrile five days after serum administration Two days later his condition was described as "bright and cheerful, much better" That afternoon he turned over in bed, developed extreme dyspnea, and died. He had suffered more than a year from chronic nephritis, arteriolar sclerosis, hypertension and heart failure and was described by his physician as "an emaciated cardiovascular-renal wreck"

Case 369 Woman, 61, ulceroglandular type Developed a small area of bronchopneumonia in the left lung on the seventh day of disease Thirty cubic centimeters of goat serum were given at once, followed by the same dose seven days later She died on the 16th day of disease Necropsy by Dr. Joseph Ganim revealed few to very few macroscopic foci of necrosis in the liver, spleen and lungs, scattered, bilateral small areas of bronchopneumonia, a dilated, hypertrophied heart with aortic valvulitis and insufficiency, syphilitic aortitis, and aneurysm of the ascending aortic arch

Case 373 Woman, 73, ulceroglandular type Had been in the hospital previously for symptoms of heart failure which had been present for three years. The heart was greatly enlarged and previous electrocardiograms had indicated coronary disease The tularemic infection was of moderate severity with no pneumonia discoverable by physical signs Horse serum, in 15 cc. doses, was given on the seventh and eighth days of disease with good effect. She became afebrile in four days and remained so There had been one attack of pain in the upper left thorax She resented complete bed rest, signed a release, and went home On the twenty second day from onset she walked out, suffered severe anginal pain, and collapsed on the street. She was taken to another hospital where she died during a second attack of anginal pain on the twenty fourth day from onset

Case 413 Woman, 30, ulceroglandular type. Subacute bacterial endocarditis had been diagnosed one year previously Infected by a market rabbit. Had bilateral primary lesions on hands, bilateral axillary buboes, and swelling of the right breast Blood culture showed a heavy growth of

Streptococcus viridans The temperature level was always near 105°F She was given 30 0 cc of horse serum on the eleventh and again on the twelfth days of disease There was no modification of the illness and death occurred on the sixteenth day of disease Necropsy showed bilateral acute hemorrhagic confluent bronchopneumonia with bilateral acute fibrinopurulent pleurisy, petechial hemorrhages in the pleurae and pericardium, acute splenic tumor, and bacterial endocarditis of the mitral valve Although six agglutination tests had been negative a bacterial skin test had been positive and lymph nodes from each axilla showed typical early lesions of tularemia

Case 478 Woman, 40, glandular type Had been known to have rheumatic heart disease for years, with chronic mitral valvulitis with insufficiency, but without evidences of congestive failure Although the infection was not severe it was decided, in view of the outcome in case 369, to give serum early in an attempt to reduce toxemia and prevent cardiac failure On the sixth day of disease 30 0 cc of goat serum were given intravenously There was marked clinical improvement, reduction in temperature, and diminution in the sizes of the involved nodes She was completely afebrile by the twenty-fifth day of disease She was kept completely at rest in bed because of fear of cardiac complications Without evidences of congestive failure she died suddenly on the thirty-fifth day from onset of tularemia Necropsy by Dr Joseph Ganum showed a dilated heart, no pneumonia, and minimal evidences of tularemic infection, insufficient to account for death

Case 509 Male, 36, ulceroglandular type, infected while rabbit hunting Goat serum was given by vein in doses of 15 0 cc on the twentieth and twenty-first days of disease of moderate severity The maximal temperature for the previous four days had been 100°F He became afebrile two days after serum administration and left the hospital improved He was seen on the twenty-sixth day of disease when he stated that he felt very well and was rapidly regaining his strength That evening he was seized with agonizing substernal pain and died within three hours Necropsy by Dr K V Kitzmiller (25) showed extensive coronary and myocardial disease with old and recent infarction, normal lungs, and insufficient tularemic lesions to account for death The hunting partner of this patient was seen one year later He told me that during the year previous to the tularemic infection the patient had had audible, labored respirations every time they scrambled up the sides of small gullies, and that

frequently the patient would have to stop because of dyspnea before he reached the top of a twenty five or thirty foot incline

Fatalities

There were 25 fatalities in the treated group. Six of these cases were cited previously and the deaths were ascribed to heart failure. In two of the cases with fatal terminations there remains some doubt concerning the establishment of tularemia as the final illness. These cases were retained in the series, as were all fatalities which occurred within one year from onset of disease, not only because serum treatment had been administered but because they illustrate certain diagnostic difficulties of the typhoidal type of the disease.

A young man, 19, went rabbit hunting several times in October, exact dates uncertain. Onset of disease on November 1, with a "cold" and sinusitis. Rales were heard in the lungs during the first few days. The temperature rose daily to 104 or 105°F. On the tenth day of disease a pleural effusion was found and two liters of fluid were aspirated. Cultures of the fluid remained sterile and a guinea pig survived for many months after inoculation with the fluid. After removal of the fluid the patient improved slowly and left the hospital late in December. He remained in bed at home for two months with occasional temperature rise to 101°F. Late in February he was well enough to get about the house. Early in March he had a sore throat with what was called influenza, a recurrence of high fever, and his general condition went rapidly downward. Chest films in April were interpreted as evidence of typical pulmonary miliary tuberculosis. Illness continued, with daily rises of temperature to 105 and 106°F, respirations 30 to 40 per minute, and pulse beats 150 to 160 per minute. On May 3 an agglutination test performed at the National Institute of Health showed agglutination of *B. tularensis* in dilution 1:320 and cross agglutination of *Br. abortus* to a lower titer. A second test, performed at the hospital laboratory ten days later, showed complete agglutination of *B. tularensis* in dilution 1:640. On the following day a controlled antiserum intradermal test was positive. This was the 196th day of disease. On that day 600 cc of goat serum were administered intravenously, followed in two days by a similar amount. After serum administration the temperature fell by lysis, about 1°F each day for six days, although there was no change in either the respiratory or the pulse rates. He died suddenly on the sixth day after serum administration, apparently from heart failure. His temperature that day had varied from

99 to 100.4°F At necropsy both lungs were studded with innumerable tubercles without any areas of extensive caseation There were numerous small infarcts in the spleen and a few larger solitary tubercles in the liver, up to 1 cm in diameter, of cartilaginous consistency, also many tiny abscesses in the cortices of the kidneys Microscopic sections showed typical tubercles Sections from two areas of the right lung, stained with carbol fuchsin, revealed typical tubercle bacilli in every miliary tubercle in the sections No lesions referable to tularemia could be found

A young man, 23, who had repeatedly hunted and dressed rabbits, fell suddenly ill with chills, fever, sweats, generalized abdominal pain, nausea and vomiting In two days there was localized tenderness and rigidity in the lower right quadrant Laparotomy revealed an acute gangrenous appendicitis The appendix was removed and drainage was established Daily remittent fever continued after operation Fluoroscopic examination of the upper abdomen, exploratory reopening of the lower abdominal incision, and roentgenologic examination of the thorax revealed no cause for the fever and the steadily downward course On the fifty-fifth day of disease his serum agglutinated *B tularensis* in dilution 1:1280 Tests against many other bacteria were negative He was given 30.0 cc of horse serum intravenously without any consequent effect upon the course of disease He became steadily worse, the lungs remained clear to x-ray examination, there was little or no drainage from the abdominal incision, and he died on the sixty-seventh day of disease Necropsy was not permitted

In the first case it was demonstrated that the patient died of generalized miliary tuberculosis The symptoms at onset were unlike the usual initial symptoms of tularemia but in rare cases patients with proven tularemia have had similar onsets, with predominant respiratory complaints and mild general symptoms It seems most probable that the positive agglutination tests and the positive antiserum test were caused by a previous unrecognized tularemic infection In the second case the cause of death was not determined but peritonitis and septicemia secondary to the gangrenous appendicitis seem probable The man was an inveterate rabbit hunter and it seems reasonable to attribute the high agglutination titer to a former tularemic infection Since diagnoses of tularemia could not be established these cases were excluded from case fatality rate computations

A third death occurred on the fifth day of disease of moderate severity, the typhoidal type with no symptoms or physical signs of pneumonia, in a woman of 62. She had cleaned a market rabbit. After an incubation period of four days there occurred an abrupt and typical onset. The diagnosis was based on the history of exposure, the incubation period, the nature of the onset and early course, and a positive bacterial skin test on the fifth day of disease. She had not had tularemia previously. The heart sounds were poor in quality and there were occasional ventricular premature beats, otherwise no localized findings of note. There was a history of cerebral embolism or thrombosis two months previous to this illness. About two hours after the intravenous administration of 150 cc. of restored lyophile horse serum the patient suddenly died. Both ophthalmic and intradermal tests with normal horse serum were clearly negative for 30 minutes before serum was administered. No respiratory symptoms preceded death and anaphylaxis seemed to be excluded as a cause. Necropsy was not permitted. It was not clear whether death was due to heart failure or to cerebral hemorrhage. The infection was not severe. The patient was completely rational and reasonably comfortable at the time serum was given. The evidence seemed to be against death due to tularemia.

Brief synopses have been presented of two cases in which serum was first administered to patients in the dying state, and the temperature curves are shown in figures 18 and 19. The clinical conditions and the responses to serum administration were very similar in the other eight patients. The important features of these cases are summarized briefly in table 7. In the last case encephalitic symptoms made it difficult to establish the time of onset of septicemia. It seems doubtful that serum could have prolonged life for 16 days, and it is more probable that the masked onset of septicemia occurred later than the twentieth day of disease. In no other case did life persist for more than five days after serum administration or for more than seven days after the onset of septicemia.

In six cases death occurred to patients who had no signs or symptoms of septicemia at the time serum was administered. It seemed certain to all of us who saw these patients that the deaths were caused by tularemia and were therefore chargeable to failures of the

TABLE 7

Brief synopsis of eight cases in which serum was administered to patients in the dying state

CASE NUMBER	SEX	AGE	CLINICAL TYPE	DAY OF DISEASE			SERUM DOSAGE	PNEUMONIA PRESENT	REMARKS
				Septicemia occurred	Serum was given	Death occurred			
67	F	62	Ul-gl	13	15	20	cc. 60	Yes	Pneumonia first detectable by physical signs three days before death. First serum given lowered temperature and dispelled delirium for a few hours. No effect from subsequent injections.
89	M	61	Typh	10	15	15	30	Yes	Enteritis with severe diarrhea soon after onset. Pneumonia first detectable by physical signs two days before death. Described as "emaciated and moribund" at time serum was given.
194	M	43	Typh	25	27	32	90	Yes	Necropsy showed typical findings of septicemia.
431	F	65	Ul-gl.	3	6	7	30	No	Vomited from onset. Had slightly enlarged heart with rales at both bases. Complicated by an acute exacerbation of chronic cholecystitis.
448	M	47	Ul-gl	1	2	11	130	Yes	Septicemia from day of onset, with continuous delirium, vomiting and diarrhea. Early pneumonia in entire right lung (x-ray). Great abdominal distension. Progressive enlargement of liver and spleen. Anisocoria. Necropsy showed typical findings of septicemia.
529	M	58	Ul-gl	11	16	17	15	Small areas	Necropsy showed bronchopneumonia, miliary foci in liver. Dilated hypertrophied heart with extensive coronary sclerosis and narrowing of lumina, but no occlusions.
551	F	44	Ul-gl.	8	12	14	60	No	Primary lesion incised on 6th day of disease. Rapidly downward course began on 8th day of disease.
562	F	64	Ul-gl	20?	22	36	60	Yes	Pneumonia early. Right lung entirely consolidated by 5th day of disease (x-ray). Symptoms of focal encephalitis in second week. Stuporous from 17th day to death.

treatment A critical review of these cases indicates in retrospect that certain of these deaths might have been prevented if the management had been different These cases are presented to indicate the aspects of management which are now considered to have been faulty and to indicate the changes which might prevent fatal outcomes in similar cases in the future There was no doubt about the diagnosis of tularemia in any case

Case 17 Male, 37, ulceroglandular type, infected by snowshoe hares Agglutination test negative on the seventh day of disease, positive in dilution 1:80 on the ninth day Scattered moist râles appeared in both lungs during the second week, without cough or physical signs of pneumonia On the seventeenth day of disease he developed severe pulmonary edema which lasted for five hours During this time the liver was enlarged, tender, and painful The spleen was not palpated After recovery from the acute pulmonary edema the liver receded to normal size and location Temperature fluctuated between 102 and 104°F Two intravenous injections of goat serum, of 12.5 cc each, were given on the twentieth and twenty-third days For three days after serum administration the temperature varied between 99 and 101°F and there was appreciable general improvement Then fever recurred to the former level, associated with daily drenching sweats On the twenty-fourth day urinary signs of nephritis appeared which steadily grew worse until the albumen was described as "almost solid by Esbach's test" on the thirty-fourth day Delirium occurred on the twenty-sixth day and gradually became continuous until death occurred on the thirty-sixth day of disease The liver did not re-enlarge and the spleen remained impalpable throughout the illness The primary lesion did not heal

Case 18 Male, 32, typhoidal clinical type Ten agglutination tests between the sixteenth and twenty-ninth days of disease gave ascending titers ranging from 1:320 to 1:5600 Pneumonia was present on the twelfth day of disease and gradually increased in size The patient was profoundly prostrated, irrational and incontinent of urine and feces Goat serum was administered in two intravenous injections of 12.0 cc each on the twentieth and twenty-fourth days, followed by very slight and brief evidences of useful effect Pneumonic areas continued to enlarge, a pleural effusion developed from which *B. tularensis* was recovered, signs of central nervous system involvement appeared and the patient died on the thirtieth day of disease This case, with necropsy findings, has been reported in detail (20)

Case 202 Male, 53, ulceroglandular type Severely prostrated at onset with temperature 104°F Horse serum was given intravenously in doses of 15 0 cc each on the sixth and seventh days of illness, followed by symptomatic improvement and a fall in temperature to 99 4°F Two days later the temperature rose to 102, prostration recurred, with accompanying cough, icterus, delirium, and enlargement of the liver and spleen On the eleventh day of illness I saw the patient, comatose, deeply jaundiced, with both liver and spleen readily felt at the level of the umbilicus, consolidation of the right lower lobe with other smaller areas of consolidation in both lungs Although he was obviously in the dying state we gave at once 120 cc of horse serum intravenously, followed in four hours by an additional 120 cc, and 30 cc six hours later The jaundice deepened very rapidly, he remained comatose to semi-comatose and died late that night at the beginning of the twelfth day of disease About 0 04 cc of blood were withdrawn from a vein just before giving the first large injection of serum This was spread over the surface of a blood glucose cystine agar slant After 48 hours of incubation there were about 320 colonies of *B tularensis* on the slant By rough calculation there were approximately 8000 colonies per cubic centimeter of blood

Case 243 Male, 60, ulceroglandular type with cough at onset and pneumonia involving the entire left lower lobe by the twentieth day of disease Horse serum was given intravenously in doses of 15, 10, 15 and 15 cc, respectively, on the 20th, 21st, 26th, and 28th days of disease Following the first two injections of serum the temperature fell from 102°F to normal during the next four days It remained normal for one day and then fever recurred Despite the additional serum injections there was a continuous febrile course from the 25th day of disease onwards, the temperature varying between 100 and 105 6°F, and the pulse from 76 to 160 per minute Previously the pulse had averaged 90 beats per minute There was at no time any diminution in the size of the axillary nodes There was no urticarial or erythematous eruption at any time On the thirty-second day he developed a cystitis which was treated by frequent 1 7000 potassium permanganate irrigations through an indwelling catheter On the forty-first day phlebitis occurred in the left leg Physical signs and roentgenologic studies showed little change in the pneumonia areas The course was steadily downward with death on the fifty-second day of disease The patient had had acute rheumatic fever as a youth with a residual mitral insufficiency of no physiologic significance At the time he acquired

tularemia he had generalized arteriosclerosis and the heart lesion then was attributed to sclerotic disease

Case 482 Male, 39, ulceroglandular type Severe onset with abdominal cramps, pain in the left upper quadrant, vomiting of blood and mucus, and great abdominal distension which continued until death occurred The temperature remained irregular with daily peaks from 101 to 105°F Signs of pneumonia appeared on the tenth day of disease and progressed steadily By the 17th day of disease roentgenograms showed irregular areas of consolidation throughout both lung fields and an effusion on the left. The sputum was purulent and pneumococci were conspicuously absent. Intravenous injections of goat serum, in 300 cc amounts, were given on the ninth and tenth days of illness, followed by slight and transient lowering of temperature but little or no effect on the abdominal symptoms or on the progress of the pneumonia He became suddenly cyanotic and dyspneic on the seventeenth day and died early the next day

Case 501 Negro male, 63, ulceroglandular type Had generalized arteriosclerosis with moderate hypertension and moderate left ventricular enlargement No symptoms or signs of pneumonia until the thirteenth day of disease From that day repeated chest films showed infiltration at the right base with progressive enlargement of the consolidated area Intravenous injections of 150 cc of restored lyophile horse serum were given on the eleventh and twelfth days of disease. The temperature curve promptly became less remittent but there was no reduction in the daily maximal peaks During the succeeding six days the patient showed marked symptomatic improvement but the temperature remained high at the previous level of about 104°F There then occurred an alternation of periods of drenching sweats and profound weakness with periods of comfort This condition was interpreted by me as an expression of serum sickness without exanthem, an opinion not held by most of the attending physicians Additional serum was not given On the twenty fourth day of disease the fever abated somewhat and the patient felt better even though the area of pneumonia was increasing in size. He insisted upon leaving the hospital, signed a release, and went home where he died on the thirtieth day of disease

DISCUSSION

Although there was evidence of heart disease in cases 17, 243, and 501, it seemed fairly clear that the deaths were caused by tularemia,

as they were in the other three cases. In cases 18, 202, and 482 there were clinical, necropsy or cultural evidences of septicemia. Although the pathogenesis and mode of death in case 17 are not clear it seems probable that septicemia occurred six or seven days prior to death. These were the only patients out of the 600 in whom septicemia developed after serum had been administered. In cases 243 and 501 death appeared to be caused chiefly by pneumonia and intoxication. None of these six patients showed a good therapeutic response with respect to temperature and general symptoms within 72 hours after serum was administered. The serum seemed to be either very low in potency or ineffective in the dosages that were used. In cases 17 and 18 the amounts administered were less than that which was later adopted as the minimal for cases of average severity *without* pneumonia. In case 202 the second 15 cc dose was given on the same day that characteristic signs and symptoms of septicemia occurred. The additional 270 cc were not administered until four days later when the patient was in coma. In cases 243 and 482 the initial dosage followed the most recent recommendation for patients with pneumonia. It cannot be said that these patients would have survived if more serum had been given but we failed to meet a definite indication for it in that their clinical conditions were not satisfactory three days after the initial doses. The same is true in case 501. This man would have received more serum if my advice, based upon the belief that serum sickness was an important contributing factor to his continuing illness, had not been followed. As noted hereafter in a discussion on serum sickness this decision is sometimes a difficult one to make correctly. Since other patients with evidences of more extensive and severer infections recovered after larger amounts of these same serums had been given at the outset within a two or three day administration period, as illustrated in figures 17 and 20, it seems possible that some of the above fatalities might have been prevented if more serum had been given during the initial administration periods.

Although the typhoidal clinical type has the worst prognosis and the highest type fatality rate only five deaths occurred in patients with this type and, as noted above, one of these was caused by a cerebral vascular accident. On the basis of previous mortality stud-

ies (7) ten or eleven deaths from this type would have been expected in this series

EFFECT OF SERUM ADMINISTRATION ON THE RESULTS OF DIAGNOSTIC PROCEDURES

Bacterial Antigen Intradermal Test

Serum was administered to 81 patients within 48 hours after intradermal injections of bacterial skin test suspensions were made. In 32 patients this resulted in suppression of the skin reaction, usually completely, or to such a degree that the very minute areas of erythema were judged to be negative reactions. Twenty of these patients were retested five to fifteen days later and sixteen of them then showed typically positive reactions. If suppurative adenitis was present the skin reactions always became positive but when suppuration was not present most of the skin reactions were negative on both tests.

Antiserum Intradermal Test

Serum was administered to 102 patients shortly after controlled antiserum skin tests had given positive reactions. Retesting of these patients either immediately or during the first 48 hours after serum administration resulted in complete suppression of skin reactions in all but a few of the most severely ill patients who had not received adequate treatment in accord with the most recently adopted scheme of serum dosage. The suppression of positive reactions was interpreted as evidence of a reversal from the antigenic to the antibody phase of infection caused by therapeutic administration of excess antibody. When the tests were again performed three to four days after serum injection positive reactions again occurred. During the first two years of the study this test was used as a very rough index of the adequacy of serum dosage for a period of four or five days after initial serum administration. The mechanism of the reaction is believed to be simply the reverse of that reported by Abernethy in relation to pneumococcus infection and skin tests (26). This use of the serum test as an index of optimal serum administration was not continued because it was found that certain clinical criteria were more reliable.

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Agglutination Test

In 135 cases in which agglutination tests were performed before serum had been given one or more tests were performed after serum administration. In 116 cases there were observed no departures from the usual titer curves shown by untreated patients. In two patients there occurred unusually high titers after administration, one rising from 1:1280 to 1:10,240 six days after serum by vein, the other rising from 1:320 to 1:80,560+ during the next thirty-three days after serum was given. In three patients who were treated during the fourth month of illness there occurred a loss of previously present serum agglutinin titers. The first patient had a titer of 1:160 on the thirteenth day of disease. No further tests were made until after serum was given on the 113th day of disease when, on the 127th day of disease, repetition of the test showed no agglutination whatever from dilution 1:10 upwards. The second patient had two positive tests, each to dilution 1:80, on the 77th and 81st days of disease. Serum was given on the 98th day of disease. On the 131st day there was no agglutination whatever from dilution 1:10 upwards. The third instance has been reported by Nelson (27). There was complete agglutination to 1:40 on the 64th and 83rd days of disease. Serum was given on the 98th day. There was agglutination only to 1:20 on the 100th day, and none at all on the 108th day of disease. In ten patients a significant fall in titer occurred after serum administration. In one of these a secondary rise occurred after the fall, and in another a rise occurred before the fall. In the first case a titer of 1:640, on the eighteenth day of disease when serum was given, fell to 1:40 seventy-six days later. Another of 1:160 on the fourteenth day* fell to 1:40 eighty-seven days later. A titer of 1:80 on the ninth day of disease was still only 1:80 sixty days later. One of 1:160 on the sixteenth day rose abruptly after serum to 1:1280 on the twentieth day and fell to 1:160 on the sixty-seventh day. One of 1:320 on the twentieth day fell to 1:40 with prezone inhibition in 1:10 on the forty-fourth day. Another of 1:1280 on the twenty-first day fell to 1:320 on the twenty-sixth day. One of 1:40 on the ninth

* In the examples that follow the preadministration titers were determined on the day of disease upon which serum was administered unless it is noted otherwise.

day, with serum given on the sixteenth day, fell to 1 20 on the twenty-fourth day. Another of 1 640 on the twenty-fourth day, with serum given on the twenty sixth day, fell to 1 160 on the thirty-second day and was still 1 160 on the forty-sixth day. Titers of 1 1280 and 1 2560 on alleged tenth and eleventh days, with serum given on the fourteenth day, fell to 1 640 on the twentieth day. The final patient had a titer of 1 10 on the eighth day, with serum given on that day. Agglutination was completely absent on the twenty seventh day and present to 1 320 on the fifty-seventh day.

In four cases there was apparently complete suppression of agglutinin formation. Serum was administered to each patient before agglutinins were expected to be present. Although *B tularensis* was not recovered from any of these patients it is believed that all had acute tularemia. The diagnoses were based upon definite histories of exposure, appropriate incubation periods, typical onsets and clinical courses, the appearance of primary lesions and regional buboes in all cases with suppuration of buboes in two, positive bacterial suspension and antiserum test reactions, and characteristic responses to serum treatment including prompt lowering of temperature and reduction in the sizes of the buboes. The data pertaining to agglutination tests and serum administration in days of disease were as follows: negative agglutination on the eighth day, serum administration on the tenth day, and no agglutination on the sixty-seventh day, no agglutination on the third day, serum administration on the sixth day, and no agglutination on the seventh, ninth or tenth days, nor four and one half years after onset of disease, no agglutination on the fourth day, serum administration on the sixth day, and no agglutination on the eleventh, eighteenth or twenty-second days, no agglutination on the seventh day, serum administration on the eighth day, and no agglutination on the twenty-fifth day.

In three cases guinea pig inoculations with exudates taken from primary lesions on the first, second and third days after serum administration resulted in death to all animals within five days and recovery of *B tularensis* by culture of the heart blood from each. Although each patient showed a significant fall in temperature, marked symptomatic relief, and considerable diminution in the sizes of the buboes on the third day after serum administration there was appar-

ently by that time no deleterious effect on the bacteria in the primary lesions and no diminution of their virulence for guinea pigs

SERUM SICKNESS

Serum sickness occurred in 309 patients, an incidence of 51.5 per cent. In most instances the severity was mild to moderate, but 97 patients (16 per cent) suffered severely. Although the same schedule of dosage was used for each serum, goat serum provoked less serum sickness than horse serum. Furthermore, the severe form was produced twice as frequently by horse serum as by goat serum. These data are summarized in table 8. A few patients had serum sickness of unusual severity with sustained high fever of 104 to 105°F for as

TABLE 8

Comparative incidence of serum sickness, and of severe serum sickness, from goat serum and from horse serum

SERUM	NUMBER OF PATIENTS	NUMBER WITH SERUM SICKNESS	INCIDENCE	NUMBER WITH SEVERE SERUM SICKNESS	INCIDENCE
			<i>per cent</i>		<i>per cent</i>
Goat	214	99	46	19	9
Horse	386	210	54	78	20
Total	600	309	51.5	97	16

long as ten to fifteen days. One suffered a cervical radiculitis with atrophy of both deltoid muscles and of parts of each scapular group.

Not infrequently the symptoms of serum sickness were so distressing that they were as bad as, or even worse than those of the disease. With respect to horse serum the fear of provoking severe serum sickness grew so strong that several of us hesitated many times to use it for this reason alone, except for severely ill patients. During the past two years the continued therapeutic and prophylactic use of histaminase for serum sickness (28) has proved so effective in preventing or controlling the disorder that we no longer have any qualms whatever with regard to giving any amount of any serum that might be indicated.

Whenever serum sickness occurred there was almost always a prompt reenlargement of all involved lymph nodes. In more than

80 cases the symptoms of serum sickness, fever, headache, backache, myalgias, arthralgias, sweats, and lymph node enlargement, were misinterpreted as symptoms of a relapse of tularemic infection. Relapses rarely occurred during the first week following serum administration, the time when serum sickness occurred most frequently, and the few that occurred were all "therapeutic relapses" due to release from serum effect when initial dosage was less than that indicated by the condition of the patient. The administration of serum in better dosage at the outset, hereinafter recommended, should prevent almost all such difficulty in the future. Since many physicians insisted upon giving more serum at the time serum sickness occurred it became very important to determine accurately the exact cause of symptoms as it is usually unnecessary, unwise, and occasionally very dangerous to administer more serum of the same animal species at this time. Critical examination of the patient will usually indicate the true nature of the disturbance. The patient himself can very often differentiate between the two groups of similar symptoms, usually, I think, because the peculiar and indescribable mental depression that accompanies tularemic disease or exacerbations is almost always absent in serum sickness. In many instances an intradermal test with normal serum will help to make the distinction. This is especially indicated when erythema and urticaria fail to appear. Finally, whenever the distinction is still a matter of doubt, two or three intramuscular injections of histaminase at hourly intervals can be relied upon to ameliorate greatly or to dispel all symptoms within six or seven hours if they are caused by serum sickness.

The likelihood of misinterpretation of symptoms caused by serum sickness and the danger of administration of additional serum during its presence were causes for considerable anxiety during the conduct of these studies, especially after additional horse serum could be obtained from trade sources. These matters are apparently not well apprehended by many who administer unrefined serums. For this reason the following case is cited.

A healthy adult acquired the ulceroglandular type of tularemia while rabbit hunting. During the second week of disease he developed bronchopneumonia with cough and hemorrhagic sputum. Agglutinin titers rose from 1:20 to a maximal of 1:1280. On the

nineteenth day of disease he was given 30 0 cc of horse serum intravenously, with additional injections of 15 0 cc each on the twenty-third and twenty-fifth days. There was marked clinical improvement which lasted until the eighth day after the first serum injection. At this time there was recurrence of fever, malaise, arthralgias, myalgias, headache, backache, lymph node enlargement, and profuse sweating. A few small scattered urticarial wheals and patches of erythema accompanied these symptoms. Despite these evidences of serum sickness the physician in charge decided that the patient had a relapse of the infection even though serum had been given in adequate dosage. He therefore gave intravenously a fourth injection of 15 0 cc of horse serum. There occurred promptly a severe chill which lasted for 40 minutes, accompanied by cough, dyspnea, high fever, and involuntary defecation. The symptoms increased rapidly in severity with the addition of neck rigidity, profound psychotic symptoms, and complete refusal to take nourishment. This condition persisted for five days with marked loss of weight and the gradual supervention of symptoms of radiculitis involving both arms. The encephalitic and radiculitic residuals necessitated a prolonged convalescence of ten months before his usual work could be resumed.

If proper differentiation between serum sickness and tularemic relapse had been made the unnecessary fourth injection of serum would not have been given and the probability is very great that the serum sickness would not have progressed to such a degree of severity. Also, if goat serum had been used for the fourth injection it is very probable that the meningo-encephalo-radiculitis would not have occurred.

STATISTICAL ANALYSES

Statistical studies confirmed and, in some respects, amplified the clinical evidence that serum therapy modified favorably the course of the disease with respect to morbidity and to mortality.

The upper part of table 9 shows the means, with their respective probable errors, for the durations of each of the selected measurable phases of the disease in the control and in the treated groups. In addition there are shown for the treated groups the means for the duration of the interval between the time serum was administered

and the time complete recovery occurred, also the means for the day of disease upon which serum was given. The lower part of the table shows the differences observed between the means for the control group and those for each of the treated groups, also the quotients of $K/P E_k$ which indicate the statistical significance of these differences

TABLE 9
Comparison of means from control and treated groups

	UNTREATED N = 518	ALL TREATED N = 600	TREATED BEFORE THE 13TH DAY OF DISEASE N = 191	TREATED ON OR AFTER THE 13TH DAY OF DISEASE N = 409
Duration of				
Disease (mo)	3 80 \pm 0 08	2 76 \pm 0 05	2 05 \pm 0 08	2 97 \pm 0 06
Disability (mo)	3 36 \pm 0 08	2 22 \pm 0 04	1 73 \pm 0 06	2 42 \pm 0 06
Adenopathy (mo)	3 36 \pm 0 12	2 45 \pm 0 05	2 19 \pm 0 08	2 60 \pm 0 06
Fever (days)	31 59 \pm 0 99	26 43 \pm 0 58	21 00 \pm 0 56	29 41 \pm 0 82
Primary lesion (days)	39 48 \pm 1 89	31 74 \pm 0 59	23 81 \pm 0 85	34 91 \pm 0 75
Hospitalization (days)	31 15 \pm 1 31	23 98 \pm 0 60	21 33 \pm 0 81	25 18 \pm 0 78
Serum-to-recovery interval (mo)		1 83 \pm 0 04	1 95 \pm 0 07	1 80 \pm 0 06
Day of disease serum was given		26 18 \pm 0 70	6 91 \pm 0 26	34 44 \pm 0 90

Significance of differences between means of control group and

	ALL TREATED DIFFERENCE	DIF. P. E. DIF.	EARLY TREATED DIFFERENCE	DIF. P. E. DIF.	LATE TREATED DIFFERENCE	DIF. P. E. DIF.
Duration of						
Disease	1 03 \pm 0 09	<i>11 22</i>	1 75 \pm 0 11	<i>15 69</i>	0 83 \pm 0 10	<i>8 46</i>
Disability	1 14 \pm 0 09	<i>12 46</i>	1 63 \pm 0 10	<i>16 46</i>	0 94 \pm 0 10	<i>9 54</i>
Adenopathy	0 91 \pm 0 13	<i>7 27</i>	1 17 \pm 0 14	<i>8 41</i>	0 76 \pm 0 13	<i>5 85</i>
Fever	5 16 \pm 1 15	<i>4 49</i>	10 59 \pm 1 14	<i>9 28</i>	2 18 \pm 1 29	<i>1 70</i>
Primary lesion	7 73 \pm 1 98	<i>3 90</i>	15 67 \pm 2 08	<i>7 54</i>	4 57 \pm 2 04	<i>2 24</i>
Hospitalization	7 17 \pm 1 44	<i>4 99</i>	9 83 \pm 1 54	<i>6 40</i>	5 97 \pm 1 15	<i>5 20</i>

The italic figures indicate statistically significant differences

The left hand column shows that statistically significant differences occurred between the means of the control group and the entire treated group for all morbidity phases except the duration of primary lesions, and that this difference just failed to attain significance. The center column shows that all of the differences between the control and the early treated groups were highly significant. The right hand

column shows that although serum was not given until the thirty-fourth mean day of the disease to the patients in the late treated group all the differences between the means of this group and those of the control group were significant except those for duration of primary lesion and duration of fever

It may be noted here that it was not until the sixth year of the study that the annual analyses showed significant differences between the means for duration of fever of the control and the total treated

Relation of Duration of Disease to Time of Serum Administration

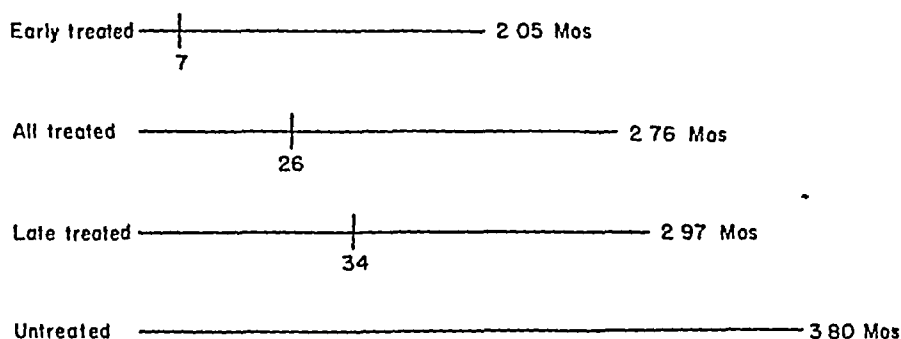


FIG 21 The diagram shows the advantage of early serum treatment in relation to duration of disease. The lines are drawn to scale and show the mean duration of disease, in months, for all groups. The mean day of disease upon which serum was administered is indicated for the treated groups by the vertical lines and numerals. To the right of these cross lines the horizontal lines indicate the serum-to-recovery intervals, to the left they indicate the durations of disease before serum was administered. Prolongation of morbidity beyond two months was almost directly proportional to the time that was lost between the seventh day of disease and the day upon which serum was administered.

groups. A comparison of the yearly figures for the mean day of disease upon which serum was administered indicates that this change was probably due entirely to the addition to the treated group of a large number of patients who were treated within the first ten days of illness during the 1936 local endemic outbreak.

A striking feature shown in the table is the almost equal duration of the serum-to-recovery intervals of the total treated group and the component subgroups. The significance of this is better shown in the linear diagram in figure 21. It required slightly less than two months for serum therapy to effect complete recoveries, regardless of whether

serum was administered on the seventh, twenty-sixth or thirty-fourth mean days of disease. Hence morbidity was definitely shortened in the subgroup in which treatment was given during the first twelve days of disease.

Additional evidence of the importance and usefulness of early treatment is presented in table 10, which shows the differences between the means of the early and late treated subgroups, their respective probable errors, and the indices of significance. The difference with the greatest significance is that between the mean days of disease upon which serum was administered. There were also highly significant

TABLE 10

Significance of differences observed between the means of the early and late treated groups

	DIFFERENCE	$\frac{\text{DIFF}}{\text{P.E. DIFF}}$
Duration of		
Disease	0 92 \pm 0 10	9 48
Disability	0 69 \pm 0 08	8 49
Adenopathy	0 41 \pm 0 10	4 23
Fever	8 41 \pm 0 10	8 50
Primary lesion	11 10 \pm 1 14	9 76
Hospitalization	3 85 \pm 1 12	3 43
Serum to-recovery interval	0 16 \pm 0 09	1 79
Day of disease serum was given	27 53 \pm 0 94	29 26

The italic figures indicate statistically significant differences.

differences for all of the phases of morbidity studied except the bed ridden period.

Since serum was used from two animal species, and since no clinical evidences of difference in relative effectiveness were noted, it seemed advisable to see if such differences could be discovered by the statistical method. The means for 197 cases treated with goat serum and for 377 cases treated with horse serum with their respective probable errors, and indices of significance are shown in table 11. The only morbidity differences that attain significance are those between duration for primary lesions and for bed ridden periods but it will be noted that the greatest difference, and the one with the highest degree of significance, is that between the mean days of disease upon which serum was administered. It happened by chance that the pa-

tients that received goat serum were treated ten days earlier than those that received horse serum. This seems to account adequately for the two significant differences, especially since the eight day difference that was observed between the mean days of disease upon which serum was given to the total treated group and the late treated group, illustrated in figure 21, caused significant differences to occur between

TABLE 11
Comparison of means goat serum vs horse serum

	GOAT SERUM CASES N = 197	HORSE SERUM CASES N = 377
Duration of		
Disease (mo)	2 65 \pm 0 08	2 86 \pm 0 06
Disability (mo)	2 02 \pm 0 07	2 33 \pm 0 06
Adenopathy (mo)	2 36 \pm 0 07	2 50 \pm 0 06
Fever (days)	23 90 \pm 0 59	28 03 \pm 0 86
Primary lesion (days)	28 07 \pm 0 66	35 61 \pm 0 89
Hospitalization (days)	20 31 \pm 0 85	25 85 \pm 0 78
Serum-recovery interval (mo)	1 86 \pm 0 07	1 82 \pm 0 06
Day of disease serum given	19 52 \pm 0 92	29 41 \pm 0 93

Significance of differences goat serum vs horse serum

	DIFFERENCE	$\frac{\text{DIFF}}{\text{P.E DIFF}}$
Duration of		
Disease	0 21 \pm 0 10	2 10
Disability	0 31 \pm 0 09	3 42
Adenopathy	0 13 \pm 0 09	1 41
Fever	4 13 \pm 1 04	3 96
Primary lesion	7 54 \pm 1 11	6 79
Hospitalization	5 54 \pm 1 15	4 80
Serum-recovery interval	0 05 \pm 0 08	0 06
Day of disease serum given	9 89 \pm 1 30	7 59

these groups for the same aspects of morbidity. There is therefore no statistical evidence that the serum from either animal species was more or less effective than the other.

Since there were two sources for horse serum, my laboratory and a commercial laboratory, it seemed desirable to test for any differences that might be disclosed by a statistical comparison of the results obtained with each serum. No clinical differences were noted be-

tween the effectiveness of these serums. The upper half of table 12 shows the constants for these groups. The test for significance of differences between constants from these groups, in the lower half of the table, shows that no difference even approaches significance. There is no statistical evidence of any difference in effectiveness between the horse serums from these two sources. Of the 280 patients

TABLE 12

Comparison of means commercially prepared horse serum vs author's horse serum

	COMMERCIAL HORSE SERUM N = 250	AUTHOR'S HORSE SERUM N = 103
Duration of		
Disease (mo)	2.92 \pm 0.07	2.86 \pm 0.12
Disability (mo)	2.37 \pm 0.07	2.34 \pm 0.11
Adenopathy (mo)	2.51 \pm 0.07	2.55 \pm 0.13
Fever (days)	27.78 \pm 0.83	30.10 \pm 1.77
Primary lesion (days)	35.72 \pm 1.04	37.02 \pm 1.71
Hospitalization (days)	25.40 \pm 0.86	26.78 \pm 1.71
Serum-to-recovery interval (mo)	1.91 \pm 0.08	1.64 \pm 0.09
Day of disease serum was given	28.63 \pm 1.40	33.35 \pm 0.98

Significance of differences commercial horse serum vs author's horse serum

	DIFFERENCE	$\frac{\text{DIFF}}{\text{P.E. DIFF}}$
Duration of		
Disease	0.07 \pm 0.14	0.50
Disability	0.03 \pm 0.13	0.23
Adenopathy	0.04 \pm 0.15	0.27
Fever	2.33 \pm 1.96	1.19
Primary lesion	1.30 \pm 1.71	0.76
Hospitalization	1.38 \pm 1.92	0.72
Serum-to-recovery interval	0.27 \pm 0.12	2.19
Day of disease serum was given	4.72 \pm 2.42	1.95

who received commercially prepared horse serum 60 received it in the restored lyophilic form, the remainder in the usual liquid form. It will be noted that there was only a five day difference between the mean days of disease upon which serum was administered to these groups, and that this difference is not statistically significant.

Table 13 shows the comparative rates of suppurative adenitis. The first line indicates that serum treatment reduced the frequency

of suppuration of nodes, and that this reduction was greater when serum was administered early. The second line shows the rates

TABLE 13
Comparison of rates for suppurative adenitis

	CONTROL GROUP N = 477	TREATED GROUP N = 534	EARLY TREATED GROUP N = 174
Rates	0 56 \pm 0 02	0 42 \pm 0 02	0 39 \pm 0 04
	Diff = 43 \pm 0 03		Diff = 27 \pm 0 04
After deduction of cases in which suppuration occurred before serum was administered		0 23 \pm 0 02	0 33 \pm 0 04

Tests by the usual statistical methods for the stability of the rates in the upper line showed that each rate was highly stable. Tests for the significance of the observed reductions in these rates showed that both differences were highly significant. The rates in the lower line are also highly significant but less importance is attached to them since the determination of onset of suppuration sometimes involved the exercise of personal judgment.

TABLE 14
Comparison of case fatality rates

	CONTROL GROUP N = 598	TREATED GROUP N = 598	
Rates	0 060 \pm 0 010	0 038 \pm 0 007	Diff = 13 \pm 0 013
After deduction of deaths caused by cardiovascular disease		0 022 \pm 0 006	Diff = 20 \pm 0 014
After exclusion of cases in which serum was administered to patients in the dying state		0 010 \pm 0 004	Diff = 30 \pm 0 013

The rates in the upper line are stable. The test for significance of the difference between these rates showed that the difference is highly significant. Although the other rates indicated for the treated group are also highly significant little importance is attached to them for reasons which appear in the text.

after deduction of those cases in which nodal suppuration had occurred before serum was administered.

Table 14 shows a comparison of case fatality rates. The rate for the untreated disease has not been determined satisfactorily, chiefly because of inadequate reporting. I have postulated a rate of 6 per

cent which, in an opinion first expressed elsewhere (7), I believe closely approximates the true situation

The statistical studies confirm the clinical opinion that serum therapy effected significant changes in the course of the disease with respect to all of the morbidity phases that were studied. The most significant favorable modifications were observed when patients were treated before the thirteenth day of disease. The evidence for reduction in the case fatality rate is equally as strong. No emphasis is placed on the lower rates indicated in the table since the derivation of these rates involved the difficult and perhaps somewhat dubious matter of determining modes of death and causes of death. I have

TABLE 15
Distribution of cases and of deaths according to age

AGES	CASES	DEATHS	MODES
0-9 9	5	0	Age incidence, 39.79 years Age at death, 64.71 years
10-19 9	39	0	
20-29 9	113	0	
30-39 9	161	5	
40-49 9	160	5	
50-59 9	78	2	
60-69 9	36	10	
70-79 9	6	1	
Total	598	23	

attempted to present all pertinent data that were obtainable and to indicate how these tentative fatality rates might be derived if these data were interpreted in the manner indicated.

Tularemia is essentially a disease of the most active and enterprising period of life. Table 15 shows the distribution of patients according to age groups arranged in decades. More than half of the cases occurred between ages thirty and fifty, and the age that contributed most patients was the fortieth year. The largest number of deaths did not occur in the decade that gave the largest number of cases, but in the third higher one. The modes for age incidence and age at death are given. In this series it is obvious that the greatest mortality risk was for patients above sixty years.

Table 16 shows the distribution of cases and of deaths grouped according to sex. The close agreement between the theoretical expectation and the observed number of deaths for each sex indicates that in a random series of treated patients sex is not a factor in mortality.

INDICATIONS FOR SERUM TREATMENT

If reduction in duration of morbidity, lessened frequency of suppurative adenitis, and prevention or amelioration of the most frequent complications and sequelae of the infection are the end results that are desired these studies indicate that the presence of continuing symptoms of the disease is an indication for serum treatment. The results will be better if serum is administered before the thirteenth day of disease. Although I know of no other means to prevent the

TABLE 16
Distribution of cases and of deaths according to sex

SEX	NUMBER OF CASES	NUMBER OF DEATHS
Male	387	13
Female	213	10

If the observed gross case fatality rate is applied equally to both sex groups the expected number of deaths would be 15 male and 8 female.

serious consequences of certain of the less frequent complications and sequelae I hesitate to advise serum therapy as a routine measure for an infection with such a low mortality rate. It seems probable that most patients that have successfully weathered the initial acute phase, those first seen during or after the fourth week of illness, with fever gone or subsiding, and with no apparent progression of localized visceral lesions, will have little genuine need for serum. Since serum can apparently be relied upon to be effective as late as the ninth month from onset of disease it would seem better to withhold it from such patients unless evidences of persisting disease or the appearance of complications should occur within that time. At this stage of illness it is uncommon to see fresh development of pleurisy, pneumonia, pericarditis or peritonitis, although each has been observed.

Serum is indicated whenever tularemic pneumonia is present, and

the indication becomes urgent if confusion and delirium supervene. Serum is especially indicated in the typhoidal clinical type of disease, and the indication is urgent if pneumoma is present. The appearance of sustained high fever associated with delirium or other psychic changes is an extremely urgent indication for immediate serum administration.

The presence of heart disease, especially coronary or rheumatic disease, is a definite indication for serum even if the disease appears to be moderate or mild in severity. There is a greater need for serum for patients above fifty years than for younger individuals.

Serum cannot be recommended as an effective form of treatment if the infection has persisted for more than ten months.

Goat serum is preferable for patients who are hypersensitive to horse serum, also for children and young adults since they are more likely to be given horse serums for other infections in the future. Goat serum should also be selected for additional treatment whenever previous use of horse serum has resulted in recent or present serum sickness.

Serum Dosage

The following dosages were adopted to meet the above indications for treatment. Although there is considerable variation in the clinical manifestations of the disease it is believed that the recommendations which follow should serve as a reasonably adequate guide to optimal dosage. The quantities given here refer to serum in the usual liquid form. If restored lyophile serum is used the equivalent dosage is one half of that stated. The restored concentrated lyophile serum is preferred to liquid horse serum, and the contents of one vacule should be administered in one dose, not in divided doses. Since the concentration of serum protein has been doubled in the restored lyophile serum the risk of fatal anaphylaxis to a patient hypersensitive to horse protein is increased. This serum must never be given unless preliminary tests with diluted normal serum have been made.

Serum dosage for children below ten years is one third that for adults, from ten to fourteen years it is one half that of the adult dose.

For adults with illness of usual severity, without pneumonia or other visceral lesions, in either early or late stages of the disease, the

dose is 30 0 cc. Additional amounts are seldom needed but if marked amelioration of symptoms, including a definite and persisting lowering of temperature, does not occur within seventy-two hours this dose should be repeated. Patients with the typhoidal type should be watched with special care since failure to obtain a satisfactory seventy-two hour symptomatic response usually occurs when there is pneumonia present which is not detectable by physical signs. This should be investigated by chest films, and if pneumonia is disclosed an additional 30 0 cc. should be given at once. Whenever pneumonia is discoverable by physical signs alone initial dosage should be 60 0 cc., administered in 30 0 cc. doses twenty-four hours apart. If symptoms of toxemia are severe the total amount should be given at the outset. If marked improvement is not apparent in seventy-two hours an additional 30 0 cc. is indicated. Patients with delirium during the first week of disease should receive 60 0 cc. at once, and additional doses of 30 0 cc. every eight to twenty-four hours until mental clarity returns and is maintained. Even when the general indications are the same the higher frequency and the more rapid progress of visceral lesions in the typhoidal type usually necessitate twice the amount of serum that would be given to patients with any of the three bubonic types. The appearance of sustained high fever associated with cerebral symptoms should be met by immediate injection of at least 90 0 cc. of serum, and doses of 60 0 cc. to 30 0 cc. should be given every eight hours until the temperature falls and delirium disappears. The psychic state is probably the best guide to adequacy of dosage. Early treatment with twice the dosage indicated above is recommended for patients with rheumatic or coronary heart disease.

SUMMARY

The chief clinical aspects of tularemia have been presented and discussed in relation to pathogenesis, diagnosis and treatment. A summary of the experience with various laboratory methods for early diagnosis has been presented. A review of a six year experience with serum treatment, including statistical analyses of the observed data, has been presented and discussed with relation to the observed or recorded clinical, laboratory, and pathological results and findings. Serum therapy effected significant reductions in both morbidity and

mortality Since the estimate of the results of treatment was an interpretation of observations and data derived from many different sources opportunity is taken to refer to those papers dealing with the effects of serum treatment that have been published by others (29)

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INFECTIOUS MONONUCLEOSIS

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I. INTRODUCTION

In the past seven years there has been a striking reawakening of interest in infectious mononucleosis. To a great extent this renaissance of attention can be attributed to the introduction by Paul and Bunnell (136) in 1932 of the serologic diagnostic test whereby many instances of the disease, which would formerly have been entirely overlooked or only suspected, could be confirmed as examples of infectious mononucleosis. Between 1928 and 1932 there were published several excellent monographs on infectious mononucleosis, notably those by Chevallier (33) of Paris, Glanzmann (70) of Berne, Lehdorff (105) and Schwarz (165) of Vienna, and Nyfeldt (131) of Copenhagen. Tidy (186) in the 1934 Lumleian lectures reviewed the problem emphasizing the observations made in the English epidemic of 1930. It is the purpose of the author to consider the subject in the light of the broadened concept of infectious mononucleosis for which the Paul-Bunnell test has supplied such valuable proof. Consequently greatest consideration will be shown for the literature since 1932. In addition references will be made to a series of 65 sporadic cases of the malady of which 17 have been previously reported (13), most of which have been studied at the Johns Hopkins Hospital in this same period of time.

II HISTORICAL

A Earliest Description

In general, Emil Pfeiffer is accredited with the first description of this disease. Under the title "Drüsenfieber" he presented, in 1889, a surprisingly comprehensive discussion of its clinical aspects (138). He emphasized particularly the predilection for children of a general infection characterized by fever, cervical glandular swelling, and sometimes enlargement of the liver and spleen. Likewise he asserted that the glands do not suppurate, that abdominal pain may be a feature and that the angina present is not sufficiently marked to serve as the primary cause of the illness. That general glandular enlargement may occur he failed to recognize.

Since the essential clinical features of glandular fever or infectious mononucleosis,¹ namely glandular enlargement and fever are asso-

¹ These terms will be used interchangeably.

ciated with a variety of conditions, it is naturally difficult to attribute with certainty the distinction of original description to any one individual. Thus, Cantlie (32) referred to a form of idiopathic glandular enlargement seen in epidemic form in children which he reported in 1891 before the Medical Society of Hong Kong. He stressed the predominant involvement of the glands about the sternomastoid muscle, a feature that Pfeiffer had observed, noted the possible confusion with mumps when there was enlargement of submaxillary or parotid glands, and recalled that the term "peculiar form of mumps" was employed to designate the Astrakan disease in 1877. Filatow (61), in later editions of his Russian text book of diseases of children, considered that idiopathic adenitis, described by him in 1885, differed from Pfeiffer's disease in the presence of generalized glandular enlargement. Actually, however, this conception approaches more closely the one held today than does Pfeiffer's. Finally Gourichon (77), in a comprehensive inaugural dissertation, published in Paris in 1895, refers not only to the claims for priority of Filatow and Korsakow, but also to those of two other Russians, Kisel and Rauchfuss who presented a case at a meeting in St. Petersburg in 1888, under the title lymphadenitis. Not until 1896 did glandular fever appear in the American literature when West (202) described an epidemic of three years' duration localized in eastern Ohio.

B Terminology

Infectious mononucleosis is probably the most satisfactory term for this disease which is, in all likelihood infectious and which sometime in its course must be associated with an increase of mononuclear cells in the blood. On the other hand neither fever nor glandular enlargement being essential signs, glandular fever is a less fitting term. These two names, infectious (or infective) mononucleosis, mentioned by Baetjer (90) in 1915 but more properly introduced by Sprunt and Evans (174) in 1920, and glandular fever (*Drusenfieber* of Pfeiffer) are favored in recent literature. However many another appellation has been applied depending upon what particular aspect of the disease attracted the attention of the individual authors. Thus, it has been called lymphoid-cell angina or monocytic angina, acute benign lymphoblastosis, acute lymphadenosis, l'adénolymphoï-

dite aiguë bénigne avec hyperleucocytose modérée et forte mononucléose, or angina et polyadénite aiguës fébriles lympho-monocytosiques.

C Developmental Phases

Clinical Following the original description of infectious mononucleosis, there appeared numerous papers most of which monotonously repeated the clinical aspects of the disease. Soon, however, passing mention was made of blood counts. Gourichon (77), in 1895, noted a mild leucocytosis. Burns (28) reporting an epidemic of glandular fever in a Baltimore hospital in 1909, deserves credit for first calling attention to the changes in the differential formula of the leucocytes. In addition to a leucocytosis "the small mononuclear elements of the blood seem to be the ones principally increased."

After convalescence there seems to be a still greater relative increase in the small mononuclear elements of the blood. In this most important, but generally overlooked paper, Burns reports a case in which the mononuclear cells composed 86 per cent of the total leucocytes.

Hematological From time to time, in the first two decades of the twentieth century, unusual instances of dramatic recovery from leucemia or peculiar blood responses to infections were encountered, some of which appear to have been obvious examples of infectious mononucleosis. Türk (192), in 1907, was chagrined to discover that a young man, to whose family he had given the hopeless prognosis that the diagnosis of leucemia elicits, had soon completely recovered after the family consulted another physician. This patient's blood at one time contained 85 per cent mononuclear cells. In 1913, Cabot (31) gave the histories of two adults with glandular enlargement, mononuclear increases in the blood and, in one, a streptococcal sore throat. These cases he differentiated from leucemia by the benign course and the fact that neither developed a lymphocytosis as high as is characteristic of the latter disease. In the same year Marchand (114) reported several similar examples of adolescents with sore throat, fever, general glandular enlargement, splenomegaly and a lymphocytosis as high as 90 per cent, the course of one being complicated by a mild acute nephritis. Under the title "a case of lymphatic leucemia

with apparent cure" Ireland, Baetjer and Ruhrah (90) recorded the story of a child with a lymphocytosis so overwhelming (97.5 per cent) that even their skeptical attitude could not dissuade the authors from concluding that leukemia occasionally does not end fatally. That the cases of Cabot and Marchand were fundamentally alike was suggested in 1915 by Osler and Parkes Weber in discussing the case reported by Hall (82) of a young man who seemingly recovered from acute leukemia. This conception was given further impetus by Deussing (47) in 1918, when he encountered two children presenting the clinical picture of diphtheria without bacteriological confirmation, whose blood showed an extreme mononuclear increase. Both patients subsequently contracted diphtheria, which further confirmed Deussing's suspicion that he had been dealing with some unusual disease similar to the cases heretofore mentioned.

Sprunt and Evans (174), in 1920, crystallized the foregoing nebulous concepts by grouping these various cases with six of their own as examples of *infectious mononucleosis*. They emphasized the clinical picture as we now recognize it but in addition pointed out that changes in the blood were characterized by the presence not only of an increase of mononuclear elements but more especially by the appearance of several varieties of abnormal cells. A year later Bloedorn and Houghton (19) reported four cases of "acute benign lymphoblastosis" occurring in young adults, generally following an upper respiratory infection and frequently associated with the presence of Vincent's organisms in the mouth. That this disease closely resembled infectious mononucleosis they admitted. The subject was further integrated in the same year by Tidy and Morley (189) who felt that glandular fever and infectious mononucleosis were one and the same disease, representing a unique clinical entity. Longcope (109) accepted the identity of the two diseases with slight reservation, after presenting ten case histories of sporadic infectious mononucleosis with particular emphasis upon the hematological abnormalities and microscopic appearance of excised lymph-nodes. Downey and McKinlay (49) studied the blood changes in infectious mononucleosis in such minute detail that little of importance in the strict hematological sense has since been added.

Once more the progress of the understanding of the disease lan-

guished behind a cloud of discussion concerning the identity of monocytic angina, lymphoid cell angina, glandular fever and infectious mononucleosis. The two last named conditions typically differ in several respects which are sufficiently striking to have provided a basis for their differentiation until quite recently. Glandular fever characteristically occurs in children's epidemics, glandular enlargement may not appear until late in its course, lymphocytosis is not as striking as in infectious mononucleosis which commonly attacks young adults in sporadic fashion with an early onset of glandular swelling. Indeed, not until recently was there serological confirmation for the conclusion that the two were fundamentally the same. Monocytic angina, a term introduced by Werner Schultz (161) in 1922, and lymphocytic angina, likewise a European concept, were at first regarded as peculiar individual hematological reactions to an ordinary throat infection, although Schultz recognized the resemblance of his cases to those of Türk, Marchand, Sprunt and Evans. Schwarz (164), in a lengthy analysis in 1929, asserted that infectious mononucleosis, glandular fever and monocytic angina were identical diseases but as late as 1934 authors felt obliged to help substantiate this view. Koegel (100), for example, reported two cases happening in his own children: the boy suffered from a sore throat whereas the girl some weeks later developed the clinical picture of a generalized infection with general glandular enlargement and splenomegaly but no sore throat. In both instances there were typical changes in the differential blood count whence Koegel concluded that his son had had monocytic angina, his daughter glandular fever, and that both were manifestations of the same fundamental process.

Serological. The third, or serological, era in the historical development of infectious mononucleosis was ushered in by the fortuitous observation, published in 1932 by Paul and Bunnell (136), that the blood serum of patients with the sporadic form of this disease may contain antibodies against sheep erythrocytes in concentrations far above a normal titer. This observation was made purely accidentally in the course of a study of non specific serological reactions in a variety of clinical conditions. So firmly entrenched among laboratory procedures has the Paul Bunnell test become, that it is now widely performed side by side with the Widal and Wassermann

reactions With the finding by Nolan (128, 129) of positive serological reactions in a number of cases of glandular fever during an epidemic in 1935 in Coronado, California, another link was added to the chain of evidence for the identity of the aforementioned maladies Final proof of this hypothesis will be reached only when the etiological agent is discovered

III DISTRIBUTION

A Age

From the earliest reports, all authors have emphasized the fact that infectious mononucleosis is a disease largely of children and young adults Indeed, with one or two exceptions (27, 189) in army ranks, epidemics have not been encountered in any group above the age of college students Of Pfeiffer's original 24 cases, 15 were under 13 years of age Again, although the epidemics studied by West occurred among a general population, all the victims were between 7 months and 13 years old Tidy and Morley (189) calculated that at least 80 per cent of all cases reported up to 1921 were under 13 years of age The apparent immunity of adults during epidemics may be quite striking as shown for example, in the well-known outbreak at the Lawrenceville School (79) where with an attendance of 500 boys, there were 112 patients sufficiently ill to be admitted to the infirmary, all but one of whom were students, whereas only one of 60 instructors was stricken Of 220 cases in Nolan's (128) California epidemic, which occurred among families of sailors of the Pacific Fleet, only 5 were adults Davis (43) described a nursery epidemic involving infants as young as 7 months of age, Price (144), likewise, encountered a case in a baby of 7 months Tidy and Morley (189) refer to an infant of 4 months mentioned by Schaffer (155), but no such case appears in the reference given At the other end of the scale there have been patients 50 years old (39), 69 (51) and even 70 years (125) of age This last case occurred during an epidemic in the Falkland Islands in which 10 per cent of the sufferers were between 45 and 65 years old, indicating the susceptibility, in an isolated community, of older people to an infection of childhood The morbidity among some adult immigrants was low whereas that among the natives was high In our cases, encountered among the population in and about a general

hospital as well as in private practice, the youngest was 6 years, the oldest 36, 81 per cent being between the ages of 15 and 30 years

B Sex

In this same group the incidence in males and females was as three to two, identical by coincidence with the ratios in Glanzmann's (70) series of 160 cases and in Nyfeldt's (131) of 33. Other authors have confirmed the greater susceptibility of males

C Occupation, Color, Race

The apparent frequency of infectious mononucleosis among individuals working about a hospital has been overemphasized. Only one-third of our cases was limited to this group. Sporadic cases, unless studied by the aid of repeated differential blood counts, may readily be overlooked while medical students or nurses, promptly admitted to the hospital for treatment of even a trifling illness, are almost certain to be correctly diagnosed by the routine methods of examination. This explanation, too, may account for the apparent paucity of cases observed in the dispensary and public wards as well as the rarity of infectious mononucleosis among negroes, of whom not a single one appeared in our cases. Indeed but a single instance in a negro has been noted in the literature (109). The disease probably has no racial preference although in an epidemic followed at the London Hospital (74) 20 of the 27 cases occurred among Jews, a fact perhaps ascribable to the area served by the hospital.

D Geographical

Infectious mononucleosis has a world-wide distribution. In addition to America, Europe and Australia, cases have been recognized in Egypt (145), the Falkland Islands (125), Trinidad (34), the Philippines (85), Hong Kong (32) and Japan (97). In Japan there are a number of epidemic fevers whose identity with infectious mononucleosis had been suspected but not until 1937, when positive Paul Bunnell tests were demonstrated, was this relationship established for the diseases known as tosa-nettsu, tokushima nettsu and lagami-nettsu (2).

E Seasonal

Most of the epidemics have occurred in the spring (187, 74) and fall (128, 70), cases in the summer are rare (202) This is not exactly in accord with our observations in sporadic instances of the disease whose temporal distribution was as indicated in Table 1 Quite striking, in our experience, has been the regular appearance of one or more cases in the fall so that, on at least several occasions, the first patient to be admitted to the Isolation Ward, which opens early in October, was found to be suffering from infectious mononucleosis

TABLE 1
Distribution by months of cases of infectious mononucleosis

MONTH	NUMBER OF CASES	MONTH	NUMBER OF CASES
January	4	July	9
February	5	August	5
March	2	September	7
April	2	October	12
May	7	November	4
June	0	December	7

IV CLINICAL PICTURE

A Introduction

In several respects infectious mononucleosis is a rather extraordinary disease It may be so mild in its clinical course that cases are entirely overlooked Thus, for example, after a school epidemic, many students not sufficiently ill to have sought medical attention were found to have enlarged lymph nodes (79) On the other hand the malady may run a severe course with high fever over a period of many weeks, possibly even with relapses, so that the victim is incapacitated for a number of months On a modest scale, indeed, infectious mononucleosis can be said to resemble syphilis by virtue of its ability to simulate so many other diseases It may appear under the guise of a commonplace follicular tonsillitis or Vincent's stomatitis, as a case of meningitis or jaundice, as an instance of appendicitis, agranulocytic angina or acute leucemia Thus several of our cases had been given injections of pentnucleotide and one was given diphtheria antitoxin

before the proper diagnosis was suspected. Only by an appreciation of the multitude of clinical manifestations of infectious mononucleosis can more exact diagnosis be attained and improper therapy avoided.

It is this variety of clinical appearance that makes it most difficult to discuss the symptomatology of infectious mononucleosis. Until recently it was unusual for any but the classical form of the disease, as described by Pfeiffer, to be recognized. During epidemics, to be sure, certain clinical variants were appreciated but there is no question that the large majority of sporadic cases went entirely overlooked. Since no one has ever died of uncomplicated infectious mononucleosis, there was no satisfactory check on the many cases that must have been improperly classified as upper respiratory infections, influenza, and so on. Certainly there is no doubt that infectious mononucleosis is a common affliction. Otherwise it would be hard to account for the large number of sporadic cases reported in the past few years.

B Clinical Types

Certain authors have quite properly set up arbitrary classifications for the clinical types. Tidy (186) mentions three forms: the febrile, anginose and glandular. While most cases can be fitted into such a grouping, its simplicity tends to becloud an appreciation of the protean manifestations of the disease. Indeed, the unique quality of infectious mononucleosis is this variability in the presenting sign. As a result papers have been written on the "hemorrhagic form" or the "icteric form," or, following Glanzmann (70) and Chevallier (33), emphasis has been placed on the individual groups of glands involved, the thoracic, abdominal and inguinal types being noted. For the purpose of discussion we conceive of infectious mononucleosis as a generalized infection whose only essential sign is, at some time in its course, an increase in the mononuclear elements of the blood. With this as a constant finding one may proceed to a description of the many clinical features which may be present either singly or in combination with one another.

That there are any important differential characteristics between epidemic and sporadic infectious mononucleosis is unlikely. It has been said that the most typical cases are the sporadic ones in which there is usually a high mononuclear count, while in epidemics the

variations are considerable and the mononucleosis less marked (33) By this same reasoning children, who have for the most part comprised the cases encountered in epidemics, have been considered to show only moderate mononuclear increases The fallacy in this argument lies in the fact that outside of epidemic times only the most typical cases are recognized, those with dubious signs or questionable blood changes being overlooked (4) Actually, however, there is just as much variability among the sporadic cases as shown by the appropriate application of the Paul-Bunnell test

C Epidemics

Epidemics of infectious mononucleosis have been frequently recorded usually under circumstances where the victims lived in close proximity to one another They have been observed, accordingly, in colleges (206, 121), schools (79, 188, 121), foundling homes (156), individual homes (189, 202), childrens' hospital wards (43, 28), military and naval bases (128, 162, 27) and in general communities (125, 158, 187, 70)

D Period of Incubation

There is a wide divergence of opinion concerning the period of incubation It has been considered to be one day (28), 4-7 days (162), 5-12 days (11), 7 days (202), 8 days (70), 11 days (43, 188), 10-14 days (33), less than 21 days (126), 14-28 days (132) Two authors who reported incubation periods of 11 days observed cases in which but a single exposure took place, so that this figure is probably most nearly accurate

E Onset

The mode of onset of infectious mononucleosis varies widely In the majority of our cases symptoms during the first four or five days of illness were the vague constitutional complaints that accompany so many infectious diseases These were usually not sufficiently pressing to necessitate a medical consultation until several days had elapsed so that admission to the hospital, when indicated, rarely occurred before the fourth or fifth day of illness On the other hand an attack may start suddenly with a chill and rapid rise of temperature

Some cases are so mild in their course as to go entirely unrecognized. During an epidemic 25 students were observed without either symptoms of infectious mononucleosis or glandular enlargement but who, nevertheless, had a mononuclear increase in the blood (4). Mention has been made previously of a similar epidemic wherein a number of students, subjectively entirely well, were found on routine examination to have abnormally large lymph nodes (79).

F Duration of Attack

In general, the acute febrile stage of the disease lasts from 7 to 21 days. Barring relapses which may prolong the course to as long as 6 months, the duration is rarely more than 1 month. In our cases the duration of the acute phase was as follows: less than 1 week, 16 per cent, 1 to 2 weeks, 35 per cent, 2 to 3 weeks, 33 per cent, 3 to 4 weeks, 14 per cent, more than 4 weeks, 2 per cent. It should be understood, however, that glandular enlargement, splenomegaly or hematological changes may persist for months or even years. A true chronic form has not been recognized. Sprunt (171) followed an individual, who apparently remained well for 2½ years with generalized lymph node enlargement and changes in the blood suggestive of infectious mononucleosis, only to observe the subsequent development of lymphosarcoma with a fatal termination (173).

G Initial Symptoms

General. The initial symptoms of infectious mononucleosis are varied. Associated with the fever there may be chills and sweats, headache, dizziness or faintness, malaise and retroorbital aching. Irritability at the onset and during the course of the illness is often impressive. Fatigue, prostration and asthenia may seem out of proportion to the apparent severity of the illness (129), so much so that French authors speak of an "asthenic form" of the disease (5). Anorexia is common, nausea and vomiting less so. There may be present the usual symptoms of an upper respiratory infection such as rhinitis, hoarseness or cough.

Sore throat. There is general agreement that sore throat, although frequently present, is not an essential feature of infectious mononucleosis (188, 33). It may be considered, therefore, a complication

of the basic disease in the same sense that meningitis is a complication of meningococcus septicemia or mouth lesions a complication of agranulocytosis. The temporal relation of sore throat is variable; it may precede the disease, be associated with the onset or course of the illness, or make its appearance after recovery has taken place. Signs of throat infection were present in 78 per cent of McKim's cases (50), in 77 per cent of ours, in all but 3 of whom there occurred subjective complaints referable to the throat. Sore throat was of 4 types: (16) diffusely injected throat (34 per cent), follicular tonsillitis or pharyngitis (40 per cent), ulcerative pharyngitis (19 per cent), membranous pharyngitis (7 per cent).

Pain in neck Pain in the neck may occasionally herald the onset of infectious mononucleosis preceding, in one of our cases, the appearance of fever and sore throat by 12 days. Combined with stiffness of the neck, this pain may become so striking as to raise the question of meningitis. Both symptoms are probably referable to enlargement of the posterior cervical glands.

Abdominal pain In three fourths of West's cases (202) there was periumbilical pain during the course of infectious mononucleosis. Abdominal pain, rare in our cases, has led to a suspicion of appendicitis (188). Davidsohn (38) reported the history of a young man whose illness began with generalized abdominal pain later localizing in the lower portion, accompanied by constipation and tenderness in the right lower quadrant. He was saved from an unnecessary operation when a blood smear revealed a mononucleosis of 84 per cent. Davidsohn suggests that certain cases with acute abdominal symptoms, in which operation revealed an appendix which was normal except for lymphatic hyperplasia accompanied by swollen mesenteric lymph nodes, may have been instances of infectious mononucleosis. Such cases have been operated upon (74, 70) and a correct diagnosis reached during convalescence from the superfluous surgical procedure. The abdominal pain is attributed to enlarged mesenteric nodes which could be palpated in over a third of West's cases.

Gastrointestinal tract, joints Pfeiffer, in his original description, appreciated the frequency of constipation. It has been our experience that this symptom is often striking while diarrhea is rare. Arthralgia is an unusual feature in the course of infectious mononucleosis but one

of our patients, in whom pain in the knee and fever were the initial symptoms, was at first thought to have acute rheumatic fever

H Temperature

Fever, the duration of which has already been noted, usually appears promptly, may reach a peak within several days, but generally not for 4 to 8 days. It usually assumes a remittent form, with afternoon rises, disappearing by lysis although crises are not unknown (144). In one of our cases the highest temperature recorded was 99° , indeed a completely afebrile course has been observed (189). Conversely, marked elevations of temperature are common, the peaks attained in our series being as follows: 98° to 99° , 2 per cent, 99° to 100° , 8 per cent, 100° to 101° , 16 per cent, 101° to 102° , 23 per cent, 102° to 103° , 18 per cent, 103° to 104° , 33 per cent. Excessively high fever has been reported 104.8° in a youth of 20 (68), 105.3° in a woman aged 30 (109), 106° in a child of 12 (58). As a rule the temperature parallels the course of the disease. Particularly characteristic is the secondary rise after an initial drop to normal, which may appear coincident with the late onset of glandular swelling or sore throat (121). A fractional degree of fever, not uncommonly persisting after all symptoms have disappeared, can safely be disregarded insofar as the activities of the patient are concerned. Tidy (187) refers to instances in which pyrexia has lasted six months or more without any obvious recurrence of enlarged glands. In general the superficial glands in these cases are only slightly, if at all, increased in size. Involvement of internal lymph nodes has been evoked, therefore, to explain this prolonged fever as well as the abdominal disturbances by which it is often accompanied.

I Pulse

As a rule the pulse rate runs parallel with the temperature but not infrequently may be conspicuously slower. Thus, in the London epidemic of 1930, Gooding (74) remarks "The pulse rate was, in uncomplicated cases, slower than would have been expected in view of the temperature. A pulse rate of 92 with a temperature of 102° was the rule rather than the exception." Similarly pulse rates of 88 (59) or 84 (8) with temperature 103.8° , 80 with 103° (86) have been

reported In one of our cases with fever of 103.4° not only was there a bradycardia of 84 but the pulse was sufficiently dicrotic in character to suggest a tentative diagnosis of typhoid fever That a slow pulse may be a manifestation of meningeal involvement is suggested by several cases in which bradycardia accompanied abnormalities in the cerebrospinal fluid Gsell (78) reported the case of one youth who, in spite of high fever, had a pulse of 80, and of another with a pulse of 60, in both of whom the spinal fluid was under increased pressure with a pleocytosis Increased intracranial pressure is not necessarily directly responsible for the slow pulse, however, as evidenced by a case with bradycardia, pleocytosis in the spinal fluid but normal pressure (57) It may well be that in at least some of the instances of infectious mononucleosis with bradycardia there was an associated meningitis which went unrecognized

J Glands

General remarks Considerable variation has been observed in the time of appearance, size and distribution of the enlarged glands, and, indeed, even in their presence or absence Enlarged glands may be the first indication of illness, preceding fever sometimes by weeks (85) In the following case enlarged glands antedated both fever and changes in the blood (16)

A 29 year old clerk noted a tender gland in the left midcervical group On the following day there were new glands on the left side of the neck with temperature 100° Two days later with fever 102.4° , enlargement of the left cervical, supraclavicular and axillary nodes, there was a leucocytosis of 12,000, 60 per cent polymorphonuclears, 38 per cent lymphocytes, 2 per cent abnormal lymphocytes In another four days the temperature was approaching normal but now out of 12,000 leucocytes only 18 per cent were polymorphonuclears, with 76 per cent lymphocytes and 6 per cent abnormal lymphocytes Two days more and the patient was well with temperature 98° At no time was there sore throat or splenomegaly

Enlarged glands may emerge in the first ten days of fever (33) In most of Scheer's (156) cases they appeared one or two days after the onset of fever, likewise in West's (202) On the other hand glandular enlargement may not set in until late in the febrile period (79), after

18 (36) or even 25 days (11) of fever In a few of Mill's cases glandular enlargement developed after the temperature had been normal for some days (121)

Enlarged glands may appear very suddenly, in one case an occipital gland, not palpable at the onset, became the size of a small walnut in 24 hours (74) Sometimes conspicuous enlargement is noted in 12 hours or less (188) The duration of enlargement is most variable, it may be as long as a year (33), or as transitory as a day or two Most important, moreover, are instances of infectious mononucleosis without peripheral glandular enlargement at any time in their course Glanzmann (70) refers to cases with typical blood count and enlarged spleen, the "splenomegalic form," and those with blood changes, enlarged liver and spleen, the "visceral form", both without demonstrable glandular enlargement. Parkes Weber (197) encountered an adult with fever, a transitory positive Wassermann reaction, and a typical blood count, but neither enlarged glands nor spleen In a second similar case, without serological changes, there was epigastric discomfort at the onset, suggesting involvement of visceral lymph glands (198) Tidy (187) suggests that infectious mononucleosis with no glandular enlargement whatever is rare, commenting as follows "Cases are on record with severe constitutional symptoms, prolonged pyrexia and late development of lymphocytosis, but with no recognizable enlargement of the superficial glands In most of these there is evidence of involvement of mesenteric or mediastinal glands " In a recent case, associated with a false positive Wassermann and a characteristic blood smear, confirmed by the Paul-Bunnell test, physical examination was entirely negative (86) Since glandular enlargement may come on so rapidly and disappear almost as promptly, it is apparent that a moderate degree might escape detection unless sought for at frequent intervals Particularly is this true in the case of healthy young people in whom small cervical lymph nodes are so commonly palpable In several of our cases glandular enlargement was minimal but, with attention directed to the point, a few nodes were always felt In one of these, previously reported (Case 16), in which the diagnosis of infectious mononucleosis was not established until a positive Paul Bunnell test was found six weeks after recovery, "~~small glands at the angles of the jaw~~" had been

noted during the acute phase of the illness, but since no one suspected the proper diagnosis at that time this observation was entirely disregarded (13) Slight glandular enlargement can readily be overlooked during a casual examination, therefore, unless one is acquainted with the normal size of the individual's lymph nodes

Size Enlarged glands ordinarily attain a moderate size, 1 to 2 cm in diameter, but may become as large as a plum (156) They may be single or in clumps but are almost always discrete They are firm and usually there is little reaction about them Tenderness is generally minimal although in one of our cases, associated with a severe follicular tonsillitis, the cervical glands were extremely tender

Cervical Most frequently involved of all groups are the cervical glands Early observers noted the typical enlargement of the glands about the sternocleidomastoid muscle, first on the left side, subsequently on the right (202) Sometimes, however, the cervical enlargement remains unilateral but within the next few days enlarged glands usually appear in other sites The posterior occipitals, submentals, axillaries, epitrochlears and inguinals are commonly affected, while other groups are involved less often Occasionally enlarged glands appear in unusual regions

Axillary Glanzmann (70) mentions a young girl whose illness started with fever and pain in the axilla, with primary enlargement of the axillary glands to the size of a hen's egg Her cervical glands were not enlarged, which suggests a resemblance to Haberfeld's disease, an acute infection supposedly transmitted by ticks in Brazil, associated with general glandular enlargement most marked in the axillae (80)

Inguinal As long ago as 1897 attention was directed to cases of infectious mononucleosis in which inguinal glandular enlargement was the presenting sign Williams (204) remarked on certain analogies to "non-venereal bubo" and repeated the idea of etiology then in vogue, namely, that the disease was secondary to involvement of mesenteric glands through an original entrance by way of the gastrointestinal tract However, Chevallier (33) claims the distinction of first recognizing the "inguinal form" of infectious mononucleosis and it is certainly the French authors, above all others, who have stressed the occurrence of inguinal glandular enlargement as the initial mani-

festation of the disease (103, 149) They even propose a venereal transmission of the malady but, as will be noted later, it is important to differentiate this variety of infectious mononucleosis from lymphogranuloma inguinale Certainly there would have to be confirmatory serological evidence for such a case as follows (12)

A 15 year old boy with inguinal glands the size of a small hen's egg, but with no fever, general glandular enlargement or splenomegaly, had a leucocyte count of 11,260, polymorphonuclears 44 per cent, mononuclears 56 per cent.

Mediastinal That the mediastinal glands may be involved is certain, and it may be that these are occasionally affected in the absence of peripheral glandular enlargement as suggested by Tidy and Daniel (188)

A 9 year old girl apparently recovering from chicken pox suddenly developed, on the sixth day, a temperature of 104° She seemed quite ill, complained of abdominal pain and 3 days later a slight cough appeared There was no general glandular enlargement or splenomegaly but examination did show indefinite pulmonary signs consisting of dulness at both bases without change in breath sounds After 6 days of high fever her temperature fell by lysis but on the following day, a younger brother developed enlarged cervical glands and ran a course characteristic of glandular fever Both children recovered completely

The authors conclude that the unexplained febrile illness of the sister may, too, have been glandular fever, with localization in the mediastinal and mesenteric lymph nodes Chevallier (33) remarks on a "respiratory form" of infectious mononucleosis with a pertussis-like cough the presenting symptom, but does not consider that this necessarily indicates enlargement of the tracheobronchial lymph nodes In the Berne epidemic (70) there were patients with cough such a marked feature as to simulate mediastinal tuberculosis or pertussis Unilateral mediastinal glandular enlargement has been encountered (103) That actual enlargement of mediastinal glands occurs has been confirmed by roentgenological examination (7, 60, 109) In one of our patients, a boy of 6 years, there were indefinite signs of broncho pneumonia along with general glandular enlargement, splenomegaly

and a characteristic blood smear The child recovered only to return a month later without peripheral glandular enlargement but with changes still present in the blood and, once again, questionable pulmonary signs on the same side as before Unfortunately for our purpose the Paul-Bunnell test was negative in both attacks

Mesenteric Enlarged mesenteric nodes were palpated in over a third of West's (202) cases Glanzmann (70) discusses an "abdominal form" of infectious mononucleosis which, in his experience, never runs an acute course but may cause recurring pain indistinguishable from that of umbilical or gall-bladder colic Tidy (186) in several cases was able to palpate definite abdominal masses which subsequently disappeared, but considered this phenomenon a rare manifestation, a conclusion with which we are in agreement

Femoral Nolan (130) dwells upon the diagnostic value of what he terms the "nodal triangle," a group of nodes converging at the inner aspect of the knee joint, running along the great saphenous and accessory saphenous veins In this triangle there may be as many as 54 discrete nodes

Suppuration Suppuration of glands is rare but probably occurs occasionally No doubt some of the cases of suppuration reported long ago were really examples of pyogenic cervical adenitis rather than infectious mononucleosis Thus Gourichon (77) casts doubt on the diagnostic accuracy of one of his predecessors, noting that while glands had to be incised in 13 of Neumann's 26 cases, surgical treatment was required in only one of his own Chevallier (33) is in accord with the sentiment of his contemporaries that suppuration indicates a secondary infection and does not occur in uncomplicated cases However, instances of glandular suppuration have occurred during epidemics, when the diagnosis was beyond question (125), so that one must conclude that this complication does rarely happen This happening could readily be anticipated, for example, in cases associated with acute follicular tonsillitis Where bacteriological studies have been made, the offending organism in glandular suppuration has been the streptococcus, staphylococcus or influenza bacillus Such glandular complications are frequently associated with retropharyngeal abscesses, which may precede or follow them (156) Any of the other sequelae of upper respiratory infections may appear, otitis media, mastoiditis (77) or, as in one of our cases, sinusitis

K Salivary Glands

Involvement of salivary glands is rare. In 6 cases where the submaxillaries and sublinguals were affected, the former suppurated in 4, the latter in 2 (77). Certain instances have been mistaken for mumps (69), particularly when there was parotid enlargement (166). In none of our cases were the salivary glands involved. Tidy and Morley (189) question the occurrence of salivary glandular enlargement but Glanzmann (70) refers to specific instances, suggesting their resemblance to Mikulicz's syndrome. In his own cases, as well as in others which he mentions, the course of this variety of infectious mononucleosis was mild and afebrile, but the blood changes were quite characteristic. An analogy with Mikulicz's syndrome is further supported by examples of enlargement of lachrymal glands (145), which may well explain the puffy eyelids sometimes observed.

L Eyes

Puffiness of the eyelids, sometimes referable to edema of the lids (162), appeared at the onset of one of our recent cases. Although not seen as frequently, it may be as striking as is observed in trichiniasis. The only other ocular sign of note is conjunctivitis, present in two of our cases. This may be the presenting sign at the onset of infectious mononucleosis, or even the initial sign, preceding fever by four or five days. Guthrie and Pessel (79) emphasize the dry granular character of the conjunctivitis which occurred in 8 or 9 per cent of their cases. It was usually unilateral, sometimes was the only sign of the acute stage of the disease, and, when present, was endowed by them with considerable diagnostic significance. Schulz (162) found a follicular conjunctivitis during the first several days in 12 per cent of his cases. In Becker's (8) case this sign appeared at the end of the second week and was unilateral. Other authors (113) consider conjunctivitis to be a frequent concomitant of infectious mononucleosis. Glanzmann's (70) attention was frequently directed to the proper diagnosis when, noting a follicular conjunctivitis at the onset of an illness, he would discover beginning glandular enlargement. At first there may be a yellowish exudate but this soon disappears. The palpebral conjunctivae may provide a fiery red background for the discrete, punctate whitish follicles, but the bulbar membranes may be intensely inflamed as well. When unilateral, the conjunctivitis is

more likely to be on the left side Glanzmann points out a similarity in the objective signs to those of trachoma It is of interest that in a canine disease produced by the injection of B monocytogenes there are many similarities to infectious mononucleosis in man, including a severe conjunctivitis (1) Photophobia has been reported at the onset of several cases (74, 174)

M Herpes, Stomatitis

Herpes labialis is usually considered uncommon (189) although Glanzmann (70) noted its frequent occurrence in children One of several varieties of stomatitis is occasionally observed Glanzmann mentions a scorbutic-like reddening and swelling of the gums Likewise the soft palate may be diffusely injected, even in the absence of subjective symptoms There may be tiny papular lesions on the palate which become confluent, or a granular appearance to the mucous membrane of the lower lip, both of which phenomena he attributes to lymphocytic infiltration In 4 of his cases aphthous lesions were so widely distributed in the mouth that the picture was that of an aphthous stomatitis When associated with a spirochetal infection, any of the manifestations characteristic of Vincent's stomatitis may be in evidence, especially gingivitis or ulcerative stomatitis

N Pulmonary Signs

Pulmonary signs at the onset are rare One instance of collapse of the lung simulating pleural effusion has been reported (74) Pulmonary findings associated with enlargement of mediastinal glands have already been discussed, complicating pneumonia will be mentioned later

O Cardiac Involvement

Cardiac disturbances during infectious mononucleosis are unusual but certain sequelae have been reported which indicate that there may occasionally be involvement of the heart during an attack In one of Longcope's (109) patients ventricular extrasystoles were noted both clinically and by electrocardiogram, but the latter method of examination disclosed no other abnormalities Several English writers (99, 146) describe cardiac complications among a number of

cases of "epidemic cervical adenitis," a disease which the authors themselves differentiate from glandular fever. However, subsequent references to these papers in discussions of glandular fever prompt us to mention them, even though they may not strictly belong here. These cases were characterized by little or no fever, sore throat, tonsillitis and cervical glandular enlargement without general glandular enlargement or splenomegaly. In from 10 to 25 per cent of the victims, most of whom were adults, there appeared cardiac murmurs or evidence of a dilated heart at the end of the first week. The patients all recovered from the immediate attack, some with persistent murmurs, but several eventually developed subacute bacterial endocarditis. The authors felt that these cases were not instances of rheumatic fever, but since the reports antedate the era of blood counts, there is no particular proof, either, that they represent examples of infectious mononucleosis. More recently (52), in a definite case of infectious mononucleosis which terminated fatally after a complicating empyema, there was disclosed at autopsy a small rheumatic like lesion on the mitral valve although there had been no clinical evidence of a valvular lesion. Even more convincing (22) is the story of a young girl, known previously to have a normal heart, who within six weeks of recovery from infectious mononucleosis developed manifestations of myocardial insufficiency and was found to have the signs of mitral stenosis. The process, interpreted as rheumatic, progressed, so that the patient died seven years later of myocardial failure (23). Despite the scantiness of these bits of evidence one wonders whether certain of the so-called rheumatic hearts encountered in individuals with no history of rheumatic fever or its equivalents, may not date back to a seizure of infectious mononucleosis. The effect of a severe attack of infectious mononucleosis upon an individual with an already damaged heart may be to bring on acute cardiac failure (39).

P Spleen

The apparent variation in the incidence of splenic enlargement in different reports is partly referable, no doubt, to the variable persistence employed in eliciting this sign. Thus, in one group of cases (43) the spleen was palpable in 11 per cent, in another group (74) the spleen was palpable in 15 per cent, enlarged by percussion in

15 per cent more, in another (50) palpable in 42 per cent, in another palpable in 60 per cent (202) In our series the spleen was palpable in 64 per cent of the cases, in Guthrie and Pessel's (79) the spleen was palpable in practically all patients sufficiently ill to be confined to bed Tidy (186) concludes that the spleen is palpable in at least 50 per cent of cases and this is probably a fair figure The splenic enlargement, usually demonstrable by the end of the first week, may occasionally appear later The organ may remain enlarged for weeks or even months In a patient whose spleen descended 8 cm below the costal margin during the acute illness there was still demonstrable enlargement 7 years later (4) The degree of enlargement is usually moderate In our cases the edge generally descended 2 or 3 cm below the costal margin, in several instances 5 cm, and in one case an entire hand's breadth Rare examples of tremendous hypertrophy are known, the spleen reaching, in such cases, the crest of the ilium (7) Of particular interest are those instances of infectious mononucleosis in which splenomegaly is present in the absence of peripheral glandular enlargement (75) Other examples of this form have already been referred to in the section on glands

Q Liver

Cases with splenomegaly and hepatomegaly without adenopathy are recognized The liver is enlarged less commonly than the spleen, in our series, for example, in 12 per cent of the cases In one epidemic, however, the liver was palpable in 90 per cent (202), in another in 100 per cent (43) On the other hand, in only one of Gooding's 27 patients was the liver palpable (74) The edge of the organ may reach to a point 6 or 7 cm below the costal margin It has been suggested that the enlarged liver is a result of hepatitis (166) but it is usually assumed that the enlargement is caused by the mechanical obstruction of enlarged lymph nodes This may not necessarily be so, since instances of hepatomegaly without jaundice have been reported (60)

R Jaundice

Jaundice may appear in the absence of an enlarged liver This is illustrated by two cases in adults (170) in whom obstructive jaundice

and fever were the presenting features in the absence of sore throat or glandular enlargement. These symptoms persisted for several weeks, the diagnosis being confirmed by a characteristic blood smear and a positive Paul-Bunnell test. That certain instances of catarrhal jaundice with splenomegaly and lymphocytosis may actually be unrecognized examples of infectious mononucleosis is strongly suggested by such a story. Jaundice is not a frequent manifestation of infectious mononucleosis, being present in only 1 of our cases, but may become quite intense (177). The earliest case with icterus was reported in 1926 (112). This patient's illness started with nausea and profound anorexia. Within the first week sore throat, cervical glandular enlargement and fever appeared. By the end of that period the liver was enlarged and the patient presented the signs of a moderately severe obstructive jaundice which persisted for several weeks. The most comprehensive discussion of the icteric form of infectious mononucleosis is provided by de Vries (48) who classifies it in three types: (1) a form in which jaundice is the first symptom, followed subsequently by glandular enlargement. This is illustrated by the last case above noted. (2) a form, described first by Chevallier, in which jaundice appears along with glandular enlargement. (3) a form in which jaundice, with or without fever, occurs as the only symptom. Of this third type this author gives 3 cases, all afebrile, 2 of which were associated with leucopenia as low as 2,350. These were two sisters who, after 10 or 11 days of vague prodromal symptoms, became jaundiced. In the first of these two tiny cervical glands appeared after the jaundice had gone, but the second patient never developed any palpable glands. In both there was a typical differential leucocyte count as well as a positive Paul Bunnell test. The third case was that of a child who, 3 months after an attack of supposed cervical adenitis, became ill with jaundice, and on this occasion had a blood count suggestive of infectious mononucleosis. Over a year later, a third illness supervened, with general glandular enlargement, no fever, typical blood count and a Paul Bunnell test, performed for the first time, positive. De Vries proposes the hypothesis that the second attack represented the icteric form of infectious mononucleosis, the third attack the glandular form. A somewhat similar case (113) is

described in an adult, who, a month after apparent recovery from an attack of infectious mononucleosis with sore throat and general glandular enlargement, became jaundiced

S Pancreas, Thyroid

Pancreatic involvement, suggesting further an analogy with mumps, has been assumed to account for rare instances of glycosuria such as Scheer (156) encountered early in the course of 3 of his cases. Infectious mononucleosis occasionally produces thyroid enlargement (4). One example of mild transitory hyperthyroidism has been noted (134).

T Nephritis

Heubner, discussing Pfeiffer's original communication, mentioned that nephritis might be associated with infectious mononucleosis. Gourichon (44) refers to a number of early authors who confirmed this observation, among them Starck, Rauchfuss, Kisel, Hoerschelmann and Soca. Among 270 cases reported up to 1921, nephritis appeared in 6 per cent (189), a similar figure being given by Moir (125) in his 87 cases. It was present in none of our cases. Nephritis appears typically in the first or second week, indeed, hematuria may be the initial symptom of the illness (74, 188). In this early onset as well as in other respects, it differs from the nephritis accompanying streptococcal infections. Abnormal constituents of the urine are predominantly red and white blood cells and albumin, less commonly hyaline and granular casts are observed. Oliguria is exceptional (77), there is neither edema nor retention of nitrogenous products (104). Renal function remains unimpaired, the course is benign, recovery rapid (11) and invariable (8). Glanzmann (70) encountered 4 cases of hemorrhagic nephritis, in one instance the signs appearing on the second day of illness, in the others between the fifth and eighth days. He notes that albuminuria, which is usually proportionate to the hematuria, may rise as high as 3 to 4 per cent, that pyuria may sometimes be marked enough to suggest pyelitis, and that slight elevation of blood pressure and cardiac dilatation occurred in but a single case. Puffiness of the lids, which may exist in the absence of any urinary changes, is not necessarily a nephritic manifestation. It is possible that increased permeability of the renal capillaries which, as in ana-

phylactic purpura, could account for the urinary abnormalities, is merely a manifestation of the hemorrhagic diathesis that is not infrequently associated with infectious mononucleosis (70)

U Orchitis, Leukorrhea

In a single case orchitis was a complicating feature (134) In young girls during convalescence, leukorrhea uncommonly appears (70)

V Hemorrhagic Phenomena

Of considerable interest are the hemorrhagic phenomena, so much so that at least one inaugural dissertation (30) is confined to this subject and other French authors report cases of "angine à monocyte à forme hémorragique" As early as 1895 it was recognized that a severe epistaxis might usher in the disease (77) This particular symptom, the most frequent of the hemorrhagic manifestations, has been reported repeatedly (5, 125, 188, 13, 68, 74, 121) The incidence of epistaxis varies widely In some epidemics no instances have been observed, there were 2 in Moir's (125) 87 cases, 2 in our 65 cases, 8 in Tidy and Daniels' (188) 30 cases, 21 in Douthwaite's 28 cases (187) The bleeding may be profuse but usually is mild, it may appear at any time in the course of the malady, particularly at the beginning, it may recur over a period of several days In a single individual there occurred epistaxis, bleeding from the gums and tonsils, and bloody sputum, all in spite of a normal bleeding and clotting time with a negative tourniquet test (68) Conversely, in the presence of thrombocytopenia, there may be no hemorrhagic phenomena, as in the case of a young man with a history of frequent epistaxes who, peculiarly enough, had no bleeding whatever during an attack of infectious mononucleosis, in spite of a platelet count of 77,600 (35) That hematuria may be regarded as part of a hemorrhagic diathesis has been previously noted Likewise rectal bleeding has been encountered both in a child (186) and an adult (91)

Since there is a common belief that the absence of bleeding is a differential diagnostic point from leucemia, it is particularly important to emphasize not only that the above phenomena may occur in infectious mononucleosis but especially that the petechial and purpuric hemorrhages may appear in the skin and mucous membranes as well

Thus petechiae have been observed on the gums, palate (in 1 of our cases), uvula, buccal mucous membranes (30), lips (91) and conjunctivae (123). Purpuric lesions may occur anywhere on the body (187). There is a special group of cases in which features characteristic of both infectious mononucleosis and thrombocytopenic purpura co-exist. The essential data of such a case follow (13).

A 16 year old female student with the history of a bleeding diathesis, was admitted to the hospital because of epistaxis. On examination she presented the typical findings of purpura hemorrhagica: fever, purpuric lesions on the skin, a palpable liver and spleen. At no time was there general glandular enlargement. The leucocyte count was 10,000, 54 per cent polymorphonuclears, 11 per cent large lymphocytes, 29 per cent small lymphocytes, 6 per cent monocytes, the platelet count 70,000, bleeding time 25 minutes, clotting time normal. The Paul-Bunnell test was positive, the Wassermann falsely positive. After a blood transfusion the patient recovered from this acute illness but continued to have thrombocytopenia with recurring manifestations thereof. Two years later, for example, for a period of several days, she suffered from hematuria which was promptly alleviated by the administration of moccasin snake-venom.

The most reasonable interpretation of such a series of events would be that an attack of infectious mononucleosis precipitated an acute episode of bleeding in an individual with chronic thrombocytopenia. Israel (91) reports a similar instance, with normal platelet count, however, occurring in a young woman known to bleed readily, who, coincident with an attack of infectious mononucleosis, suffered from hemorrhages from the lips and rectum. Two other cases, identical with ours except for an absence of a chronic hemorrhagic diathesis, have been reported by Minot (123) and by Williams (187), indicating that acute as well as chronic thrombocytopenic purpura may appear coincident with infectious mononucleosis. Furthermore, 3 similar instances have been described under the title "purpura hemorrhagica with lymphocytosis" (124), although Minot concedes that they may represent atypical cases of infectious mononucleosis with pronounced thrombocytopenic purpura. In view of our experience above noted, even though the Paul-Bunnell test was negative in the 1 case in which it was performed, we prefer to consider these last 3 cases in the category of infectious mononucleosis with thrombocytopenia.

W Venous Thromboses

Venous thromboses are uncommon. One definite example (121), and a questionable instance (74) of femoral thrombosis, have been recorded.

X Skin

In addition to purpuric eruptions (49) already mentioned, a host of other types of skin rashes have been observed. As in so many other features, certain of the dermatological manifestations of infectious mononucleosis were recognized early in its history. Thus, Gourichon (77), quoting the experiences of some of his contemporaries, noted the occurrence of a morbilliform, an urticarial or an erythematous eruption.

A typhoid like eruption (140, 186). In Tidy's experience in the 1930 epidemic, this was present in the early stage of almost all the cases of the "febrile type" in which fever preceded glandular enlargement. This maculo papular rash appears usually between the fourth and seventh days, most commonly on the fourth or fifth but occasionally as late as the tenth. The spots are 2 to 5 mm in diameter, pink or pinkish brown in color, and may disappear on pressure. There is usually only a single crop, numbering anywhere from 8 or 10 to fairly numerous lesions, which fade in about 4 days. The lesions are confined principally to the anterior trunk, less commonly they appear on the back, limbs or face. In brief, then, this eruption closely resembles rose-spots and has led to an erroneous diagnosis of typhoid (121) or paratyphoid fever (11).

A morbilliform eruption (11, 186, 187). This may precede or follow glandular enlargement. The lesions, at first discrete, may become confluent and occasionally leave a temporary residual pigmentation. This sort of rash is said to be more common in children than adults (70), and when present may be difficult to distinguish from that of German measles.

A scarlatiniform eruption (52, 78, 145). This erythematous rash, most common on the trunk and abdomen, may be followed by desquamation and thus be confused with that of scarlet fever, particularly if sore throat is present.

A typhus like eruption (11) Radford and Rolleston (147) reported two such cases in a mother and daughter

Erythema nodosum In a case described by Lohe and Rosenfeld (107), lesions typical of this condition appeared four weeks after the onset, by which time the patient's fever had disappeared

Urticarial eruption (77) In one of our cases urticarial lesions appeared in one axilla on the second day, persisting for five days In Gooding's (74) case, this rash lasted but 24 hours

Vesicular eruption Vesicles appeared on the forearms of one of our cases on the twelfth day, just after the temperature had fallen to normal

These rashes are not always clearly differentiated so that the morbiliform or the scarlatiniform may blend into one another, resembling at some stage of their evolution an erythema multiforme The incidence of cutaneous eruptions in infectious mononucleosis is variable, in our series, for example, it was 9 per cent No doubt, with more careful and frequent observation, transitory rashes which are missed under ordinary circumstances, would be noted

Y Central Nervous System

Quite recently central nervous system abnormalities have been recognized Almost simultaneously, in 1931, Johansen (93) and Epstein and Dameshek (57) were the first to describe such changes A typical case may be cited (89)

At the onset headache was the presenting symptom Examination showed general glandular enlargement and splenomegaly, 7,600 leucocytes with a characteristic differential count The Paul-Bunnell test was positive There were 350 cells, almost all mononuclears, in the cerebrospinal fluid Two days later signs of meningitis were quite marked including extreme hyperaesthesia in both feet Lumbar puncture showed 1,000 cells with a meningeal type of mastic curve Two weeks after admission to the hospital, the patient was well

Altogether there have been at least 12 cases of infectious mononucleosis accompanied by a clinical picture of serous meningitis Often the meningeal symptoms antedate the signs of infectious mononucleosis, so that other instances, in which changes in the blood and glandular

system were not repeatedly sought for, have doubtless passed under the guise of benign lymphocytic meningitis, encephalitis, or abortive poliomyelitis. In one case (78), for example, the characteristic changes of infectious mononucleosis did not appear until the second week, some days after the abnormal changes in the spinal fluid were first determined. The commonest initial symptoms in this form of the disease are headache, the usual signs of meningeal irritation and blurring of vision. In certain instances there have been convulsions, stupor, or coma (39). The patient may be irrational and combative (57). There may be stiff neck, positive Kernig and Babinski signs. In some, but not all of these cases, there has been bradycardia. Bilateral optic neuritis, remnants of which were still visible a month later, has been described (78). Changes in the spinal fluid will be discussed in detail in the section on laboratory findings, briefly, they consist of a moderate pleocytosis, with or without increased pressure, and usually with some alteration in the protein content and mastic curve.

In one case central nervous system signs, present during an initial attack of infectious mononucleosis, recurred with increased violence during a relapse, with coma and convulsions from which the patient almost died (184). Spinal fluid changes may exist in the absence of clinical signs, conversely there may be clinical signs of meningitis in the presence of a normal spinal fluid (58, 174). Thus, in 10 patients, Huber (89) found meningitis in 3, only 2 of whom had abnormal spinal fluids while lumbar puncture in 5 others, without meningeal signs, disclosed pathological findings in at least 3. Likewise Schmidt and Nyfeldt (160) encountered abnormalities in the spinal fluid of 4 out of 5 patients, only one of whom presented the clinical signs of meningitis. In view of the high incidence of neurological changes in their cases, the diagnosis of which was confirmed by the Paul-Bunnell test, these authors propose that routine lumbar puncture be carried out in all instances of infectious mononucleosis. In an epidemic of 70 cases among children, with certain resemblances to the above described condition, pleocytosis was found in 16 of 18 patients whose spinal fluid was examined (190).

Peripheral cranial nerves are rarely affected. In one instance anosmia persisted after the patient had otherwise recovered (113).

One example of slight unilateral ptosis has been noted (57) In one case (78) a right facial palsy, appearing on the twenty-first day of illness, was followed a week later by one on the left, both disappearing completely in less than 2 weeks This same author describes a unilateral facial palsy of one week's duration, which came on a month after apparent recovery from infectious mononucleosis The significance of central nervous system involvement is succinctly stated by Epstein (56) " The crux of the whole problem lies in the larger concept of various systemic diseases of known and unknown etiology, giving rise to changes in the central nervous system I am inclined to believe that such diseases as infectious mononucleosis may produce cerebral changes, and conversely that so-called lymphocytic meningitis, like certain of the encephalitides, is merely symptomatic of some as yet unknown systemic disease "

Z Association with Other Diseases

Certain clinical conditions are sometimes associated so intimately with infectious mononucleosis as to be considered part of the disease Among these are Vincent's angina, follicular tonsillitis and purpura hemorrhagica Other diseases have occurred in association with infectious mononucleosis but to such a limited extent that the association must be regarded as coincidental In 6 of one series of 50 cases (4), there was an antecedent syphilitic infection for which the individuals were being treated at the time of onset of infectious mononucleosis In another case (60), in which enlarged inguinal nodes were referable to a chancre, seropositive primary syphilis was associated with a severe form of infectious mononucleosis Again, a case (203) appeared in a sailor under treatment for syphilis, which he had contracted two months before An army man in Egypt, under treatment for tertian malaria with quinine, atabrine and plasmoquine, developed infectious mononucleosis (145) The specific medications are mentioned since, as will be noted later, certain drugs may produce a lymphocytosis but no doubt of the correctness of the diagnosis existed here since it was confirmed by a positive Paul-Bunnell test, as it was in another instance where these two diseases occurred together (115) In a child suffering from nephrosis (7), infectious mononucleosis supervened without affecting the course of the underlying condition

Infectious mononucleosis may closely simulate typhoid fever but the two maladies have appeared simultaneously (154) In another patient (163) typhoid fever was the initial disease but within 10 days of the onset, the characteristic blood changes of infectious mononucleosis were present and the Paul Bunnell test, first performed on the fourteenth day, was positive One of West's (202) cases appeared during convalescence from scarlet fever, in 4 others the latter disease complicated recovery from infectious mononucleosis An instance of what was presumably mediastinal glandular fever (188), began on the sixth day of chicken pox. Conversely, chicken pox may begin during convalescence from infectious mononucleosis (109) In a child with a history of diphtheria three years before, a second attack came on several days after recovery from infectious mononucleosis (110) As has been observed with other complicating infections, there was, coincident with the onset of diphtheria, a prompt polymorphonucleosis with an increase from 50 per cent to 93 per cent within 2 days Two cases of infectious mononucleosis (154) occurred in the course of lymphogranuloma inguinale

Associated pulmonary diseases are of some importance since these have produced several of the rare fatalities in infectious mononucleosis Collapse of the lung at the onset has already been mentioned (74) There may be pleurisy and pneumonia (134), or pleurisy with effusion (142), appearing as complications A diffuse bronchopneumonia has been recognized as early as the third day of illness in a patient who was desperately ill but who recovered after more than five weeks (58) In another case, bilateral bronchopneumonia with a fatal termination, developed 8 days after admission to the hospital (74) A young man died less than 4 weeks after the onset of infectious mononucleosis, of bronchopneumonia and putrid empyema (52) While the purulent pleural fluid was said to be sterile, it contained occasional mononuclear cells similar to the abnormal ones found in the blood.

AA Relapse

Relapses are not uncommon In our series three patients, after becoming afebrile, had a return of fever within 3 to 7 days In one group of 9 infants (43) 5 had relapses, the interval between attacks varying from 5 to 24 days In these, although the objective signs

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during relapse were essentially the same as during the original attack, subjective symptoms were milder and the duration shorter. Occasionally the relapse is more severe than the initial episode. Usually the same glands that were originally involved become enlarged again during the relapse and the abnormal blood picture returns (33). Where cervical glandular enlargement was originally unilateral, the relapse may be accompanied by swelling of glands on the opposite side (74, 189).

BB Recurrence

Recurrences in infectious mononucleosis are rare. In one individual two attacks occurred five years apart (152). Another patient suffered four attacks in three years (4). The recurring episodes do not necessarily assume identical forms so that in one, for example, jaundice may be the presenting symptom, in another, glandular enlargement (48, 113). In one of Chevallier's cases (33) there were two attacks of inguinal and one of axillary adenopathy within seven months.

CC Contagiousness

The transmission of infectious mononucleosis presents certain puzzling features but one must conclude that its contagiousness is low. During epidemics, to be sure, multiple cases are frequently encountered in a single household. Likewise, sporadic cases may affect several individuals in one house (100, 133, 189). However in not a single instance in our group, and notably among the medical members who live and work in intimate contact with one another, could an infection be attributed to association with another patient. Cases of infectious mononucleosis have been treated in the general ward without any spread of the infection (74). Conversely, cases have appeared simultaneously in households, isolated from one another, without any obvious direct method of transmission (202). The occurrence of sudden widespread outbreaks suggests that there may be carriers.

DD Fatal Cases

In not a single case has uncomplicated infectious mononucleosis proved fatal. The benign outcome in a disease whose course can be so severe and protracted may be attributed either to the youth of its

victims or else to the variety of antibodies which may appear in response to the infective agent. Most of the fatal cases that have been reported are probably not examples of infectious mononucleosis at all, but rather of generalized sepsis with general glandular enlargement (77, 81, 103, 189) One of West's (202) patients, "a delicate child convalescing from scarlet fever," died, but no further details are given One patient died of asphyxia following a ruptured retropharyngeal abscess (125) Two fatalities are attributed to pulmonary complications, bronchopneumonia (74) and empyema (52)

EE Sequelae

A number of patients with infectious mononucleosis have been followed for many years without any sequelae of note (105) We know of one case, well 30 years after an attack One of Longcope's (109) cases developed tuberculous pleurisy six years later, a physician, not included in our group, died three years later of tuberculous meningitis There is no reason to assume that these represent anything more than coincidences that might occur in any group of young people It has been suggested that infectious mononucleosis may be an abortive benign form of acute lymphatic leukemia (63) Glanzmann (70) quotes Lenhartz's hypothesis that infectious mononucleosis may be the primary stage of a chronic disease, followed by a latent period, and terminating in chronic lymphatic leukemia, analogous to the course of syphilis with the appearance of tabes or paresis in the late stage

V LABORATORY FINDINGS

A Blood

Since the time when abnormal blood findings were first recognized, the suggestion has frequently been made that infectious mononucleosis is not a clinical entity at all, but rather a peculiar individual reaction to a number of different infectious agents Many instances of the disease have been observed, however, in which some secondary infection elicited a normal polymorphonuclear leucocytosis Accordingly, ample evidence has accumulated to indicate that this hypothesis is unfounded so that today one rarely, if ever, hears it expressed Thus, in one case a peritonsillar abscess was accompanied by an outpouring of polymorphonuclears which, when the abscess was incised, disap

peared, leaving the original lymphocytosis (53) Similar phenomena have been associated with a complicating otitis media (161), diphtheria (110), or pneumonia (134, 74) An injection of typhoid vaccine during an attack of infectious mononucleosis has produced a 'polymorphonuclear increase, with a return to the original abnormal differential count within 24 hours (4) One of Sprunt and Evans' (174) patients developed tonsillitis three months later but this time with a perfectly normal polymorphonuclear response Such a case we have encountered

A 24 year old medical student at the height of an attack of infectious mononucleosis had temperature 103.6° , general glandular enlargement, splenomegaly, sore throat, leucocyte count 7,960, polymorphonuclears 25 per cent, lymphocytes 75 per cent, positive Paul-Bunnell test, false positive Wassermann Five months after recovery he returned with sore throat again, temperature 104° , less marked general glandular enlargement, leucocyte count 7,500, polymorphonuclears 55 per cent, lymphocytes 45 per cent On this occasion the Paul-Bunnell test and the Wassermann reaction were negative

Earliest changes Before discussing the blood changes it is necessary to recall what was said earlier about the differences between epidemic and sporadic cases In all likelihood the more consistent occurrence of extreme mononucleosis in sporadic cases is due to the fact that only the most typical instances of the disease are recognized, while during epidemics even a slight deviation from the normal blood count will be sufficient to cause a search for other manifestations of the disease In infants and children the earliest change in the blood may be a polymorphonuclear increase with a shift to the left, followed later by a lymphocytosis (157) On the other hand, lymphocytosis may be present from the start as in a nursery epidemic where routine blood counts disclosed a lymphocytosis a week or more before the onset of symptoms (43) Likewise in young adults, a transient polymorphonuclear leucocytosis may appear at the onset with a subsequent lymphocytosis which sometimes does not appear until the temperature has reverted to normal (79) During the early days of one of our cases (13) not only was there a polymorphonuclear leucocytosis of 87 per cent but an absolute lymphopenia, 13 per cent of a total white blood

cell count of 6,900 This same phenomenon was observed in a second case in which, during the prodromal period, there was a polymorphonuclear increase with normal leucocyte count (16) Blood counts are usually not made until four or five days after symptoms have first appeared, by this time a well marked lymphocytosis is generally established In the great majority of cases, therefore, by the time the patient first consults a physician, there is sufficient abnormality of one sort or another in a blood smear to warrant repeated hematological examinations, which within the succeeding week will almost always indicate the correct diagnosis

Leucocyte count At some stage of the illness the total leucocyte count is usually somewhat elevated The highest levels attained in

TABLE 2
Highest leucocyte count in 65 cases of infectious mononucleosis

LEUCOCYTE COUNT	PERCENTAGE OF CASES
6- 8,000	7
8-10,000	25
10-15 000	39 5
15-20,000	22
20-25,000	3 5
25-30 000	1 5
Over 30,000	1 5

our cases, in almost a third of which they did not exceed 10,000, were as indicated in Table 2

Within the first week there may be a well-marked leucopenia due chiefly to reduction of the myeloid cells but sometimes in part referable to diminution of the mononuclear elements Thus, in one of our cases the leucocyte count on the sixth day was 2,950, polymorphonuclears 59 per cent, lymphocytes 41 per cent. Where leucopenia exists at the onset, the leucocyte count invariably rises to a normal level before fever has disappeared but seldom above that level In our 7 cases of this type with a count below 4,000, the leucocytes rose above 10,000 in only 2 Other examples of leucopenia have been noted 2,400 (203), 2,150 (20), 2,000 (186), 1,500 (39)

Extreme leucocytosis is rare, not more than 10 instances of counts over 40,000 have been recorded, all but three of these in infants or

children under 10 years of age The highest reported leucocyte count is 63,000 (44) Tidy (186) cites others of 63,000 (Feer), 58,000 (Nelson), 54,500 (Feer), 41,200 (de Lange) In one of our cases, a child of 10 years, the count was 46,000, in the 7 month infant studied by Price (144) the count was 44,200 The only adults in this group are a man of 35 with a leucocytosis of 42,000 (118), a woman of 22 with 49,000 white blood cells (6), and a man of 47 with a count of 60,000 (141) Such high counts are produced chiefly by a lymphocytic increase, the polymorphonuclears taking little or no part in the process

In general the leucocyte count parallels the clinical course of the disease A relapse, or the late appearance of sore throat, may be accompanied by a secondary rise of the white blood cell count Exceptionally, as in two of our cases, the count may reach a peak coincident with or even a day after the disappearance of fever

Myeloid cells In the presence of leucopenia, lymphocytosis, or a combination of these two factors, there may develop a neutropenia at times attaining profoundly low levels Tidy, (186) refers to cases in which the absolute number of myeloid cells fell below 1,000 750 (Glanzmann), 250 (Glanzmann), 160 (Schmidheiny) A few other examples of granulopenia are 1,200 (59), 1,020 (139), 882 (39), 777 (206), 510 (49), 368 (109), 48 (2 per cent of 2,400) (203) In two of our cases there was an extreme reduction of polymorphonuclears in one to 990 (4 per cent of 24,750), in the other to 800 (10 per cent of 8,000) Neutropenia, generally most conspicuous early in the disease, did not appear in these two until the third week of illness, in one instance the temperature having fallen to normal There may occur qualitative as well as quantitative changes in the myeloid cells, notably an increase of younger forms (58) The proportion of stab cells to segmented forms may be as high as 7:1 (177) This shift to the left may persist into early convalescence (11) York and Eckley (206) stress the diagnostic worth of the high non-filament count which is absent in many of the diseases simulating infectious mononucleosis, and emphasize the paradoxical presence of this sign which, in most maladies, indicates an unfavorable prognosis

Eosinophils As in other infectious diseases, eosinophils are typically suppressed during the acute phases In two of our cases the eosinophils were 4 and 6 per cent, in the rest usually absent, occa-

sionally 1 or 2 per cent. Glanzmann (70), however, reports an eosinophilia of 9 per cent in several instances, of 15 per cent in one. On the other hand, during convalescence the eosinophils commonly rise to 5 or 6 per cent as in several of our cases, infrequently to 9 per cent (8), to 12 per cent (118), or even 15 per cent (70)

Mononuclear cells To our mind mononucleosis at some stage of the disease is an essential sign although Chevallier (33) suggests that in some instances this may be so slight that the diagnosis must be made only on the presence of abnormal cells. Mononucleosis may exist even in the prodromal period but typically appears within the first four or five days of illness and rapidly reaches a peak within a week or ten days. In one of our cases the high point, 96 per cent, was not attained until well along in the third week, but this is exceptional

TABLE 3
Degree of mononucleosis in author's cases

PERCENTAGE OF MONONUCLEAR CELLS	PERCENTAGE OF CASES
40-50	4
50-60	9
60-70	26
70-80	33
80-90	23
Over 90	5

The maximum values attained in our cases are shown in Table 3. In the two instances of mononucleosis below 50 per cent, only single observations were available. No doubt further differential counts would have revealed a higher figure at some other time. The highest mononucleosis in these cases were 92 per cent in two, 96 per cent in one. Other high values have been reported, 92 per cent (49), 93 per cent (39), 94 per cent (11), 96 per cent (109), 97 per cent (70), 97.5 per cent (90), but these are uncommon and statistics, in general, conform to our table above.

Having reached a peak, the mononucleosis gradually diminishes over a period of several weeks or months. The excess mononuclears may disappear rapidly however, as in two of our cases. In the first, the leucocyte count was 14,150 on the eighth day with 70 per cent

mononuclears, on the fourteenth day leucocytes were 7,100, 34 per cent mononuclears, in the second the leucocyte count was 15,000 on the sixth day with 87 per cent mononuclears, on the twentieth day leucocytes were 6,800, 40 per cent mononuclears. On the other hand, a mononucleosis may persist for 9 (121) or 10 months (193). In one of our cases, 15 months later, there were still 57 per cent mononuclears in 6,300 leucocytes. Farley (60) records the remarkable story of a patient whose blood 6 years later was still abnormal, with 60 per cent mononuclears. Even 10½ years after the original illness there were observed atypical cells characteristic of infectious mononucleosis. It must be borne in mind, therefore, that a lymphocytosis, or the presence of abnormal lymphocytes in a routine blood examination may, if no other explanation is forthcoming, be evidence of an attack of infectious mononucleosis months or even years before, so that specific inquiry may be pointed in that direction.

The character and nature of the mononuclear cells have been the basis of much discussion. There are present, first of all, normal small and large lymphocytes, and monocytes. Young lymphocytes are observed, indeed, mitotic figures may be encountered in the peripheral blood (48,177), and there may rarely be a "shift to the left" of lymphocytes just as in the myeloid components (51). The specific cells which give the blood smear of infectious mononucleosis its distinctive features are probably also members of the lymphocytic series. Most of the pioneers in the morphological description of these cells favored this origin (4, 19, 188). Certain ones (4) were undecided between a lymphoid or reticulo-endothelial source, pointing out that these same cells might appear in small numbers in other conditions, most of which were associated with enlargement of the lymph nodes, such as hyperthyroidism, typhoid fever, serum sickness, diphtheria, and staphylococcal and streptococcal adenitis. Presumably, when there is hyperplasia of lymphoid tissues, these abnormal cells, when present therein, may migrate into the blood stream. That the controversy is not yet settled is indicated by an opinion expressed in 1937, that "both abnormal lymphocytes and immature monocytes occur" (91).

The abnormal or "leucocytoid" lymphocyte is variable in size, structure and staining properties, this variability being a characteristic feature. It ranges in size from that of a small lymphocyte to that of

a monocyte The nucleus, which may occupy a portion or almost all of the cell, may be round, bean-shaped or irregular, centrally or eccentrically situated, and typically darkly, but coarsely stained The cytoplasm may be light but characteristically is deeply basophilic and vacuolated or "foamy", a few azurophilic granules may be present A small proportion of these cells, as well as the normal small lymphocytes, may show fenestrations in the nuclei, an appearance produced by actual holes piercing the nucleus in various directions (133) This phenomenon may in certain instances be related to the irregularly stained quality of the nucleus Studies with supravitaly stained preparations led McLean (119) to conclude that the abnormal cells were neither lymphocytes nor monocytes but rather "lymphendotheliocytes" More recent application of the same method, however, has produced evidence for the lymphoid origin of these cells, indicating that they are relatively mature but atypical (67) In infectious mononucleosis there is a constant diminution of lymphocytes bearing refractile granules, a feature which this same author considers of great diagnostic worth

Erythrocytes Anemia of any appreciable degree does not appear unless associated with some complicating feature such as hemorrhage (91) or dietary deficiency (144) In one of our cases the hemoglobin fell to 70 per cent at the end of the illness The fragility of the erythrocytes is normal (16, 131)

Platelets In a small number of instances, platelets have been reduced Thrombocytopenia as low as 45,000 may exist in the absence of any hemorrhagic phenomena (35, 92, 127), or along with bleeding manifestations as in the cases discussed in the clinical section Conversely, a normal platelet count may accompany striking evidences of a hemorrhagic diathesis (91)

Bleeding and clotting time, tourniquet test Bleeding time may be prolonged (187) as much as 25 minutes (16) Clotting time is not disturbed (68) Only 3 instances of a positive tourniquet test have been reported, in one no platelet count was made (187), in the second platelets were normal (30), in the third platelets were 70,000 (16)

Blood groups Cases are normally distributed throughout all the blood groups (13, 177)

Blood chemistry No abnormality of the blood sugar or non protein

nitrogen has been observed. The total proteins are not elevated although in view of the presence of such a variety of antibodies as may occur one might expect an increase of the globulin fraction (16). Where jaundice is present the icteric index may rise to 50 units (177), the van den Bergh reaction may be positive, delayed (16) or direct (177) biphasic and as high as 8 mgm per 100 cc. Under such circumstances a bromsulphalein test may give evidence of impairment of hepatic function (136).

B Wassermann Reaction

The occurrence of false-positive serological tests for syphilis has been discussed at length in a recent publication (15). Such observations have been made by a number of authors (74, 86, 107, 145, 147, 195, 198, 199). In 44 of our cases, where one or another of the serologic tests for syphilis was carried out, a transitorily positive reaction was encountered in 8, an incidence of 18 per cent (15, 16). The Wassermann reaction was positive in 7, the Eagle test in 4. The Wassermann titer may run as high as 150 units. The duration of positivity is brief, usually a matter of a few days, rarely as long as three months (96). The evanescent character of these positive serologic reactions (they generally revert to negative before the end of the third week) mitigates against the likelihood of recognizing all the instances in which they occur. They are independent of the presence of sheep-cell antibodies in the patient's serum, as can be demonstrated by absorbing out the latter without reducing the Wassermann titer.

C Other Antibodies

In addition to the substances producing false-positive tests for syphilis there are a variety of bacterial antibodies which may be present. The occurrence of a false-positive Widal reaction has been noted (13, 121). The serum of one young woman, with no history of typhoid fever or typhoid vaccine, agglutinated *B. typhosus* and *B. paratyphosus* B to a titer of 1:640, and *B. paratyphosus* A 1:320 (16). In 19 of our cases where agglutination tests of one sort or another were carried out, one or more significantly positive reactions were obtained in 31 per cent. In one of these there were agglutinins against not only the enteric fever group but also *B. aertrycke*, *B. supestifer* and *B.*

enteriditis These various antibodies disappear in a few weeks or months at about the same rate of speed as do the sheep-cell agglutinins Agglutinins against *B. melitensis* have been found (13), likewise an isoagglutinin, an autoagglutinin and a rouleau-forming property (10) In general, however, isoagglutinins are not increased (40), nor is diphtheria antitoxin (194)

D Urine

Ordinarily nothing more abnormal is found in the urine than the trace of albumin that may be encountered in any febrile illness In instances of nephritis there may be gross hematuria early in the disease which returns again after an interval of quiescence Albuminuria, which is usually in proportion to the hematuria, may rise as high as 3 or 4 per cent Pyuria may be marked cylindruria less striking (70) Renal function remains unimpaired (8) Early in the course of three cases a reducing substance was found in the urine (156) Fairly large amounts of urobilin and bile may be present (16), in one series, urobilinuria was noted in half the cases (97) Acetonuria may appear (130)

E Cerebrospinal Fluid

As already mentioned, there may be changes in the cerebrospinal fluid irrespective of whether or not there are clinical signs of meningitis The pressure may be moderately elevated Pleocytosis, usually below 200, may rise to 1,000 (89), almost all the cells are mononuclears The sugar content is normal (57) The protein content may be increased and the Pandy test strongly positive (184) Inconstant abnormalities of the mastic curve are recognized (78) Even when the blood Wassermann is falsely positive, the cerebrospinal fluid Wassermann is negative (73, 107) Cultures have been negative with the exception of four cases, verified by a positive Paul-Bunnell test, from which *Listerella* were isolated (160)

F Bacteriological

Blood cultures have been uniformly negative Nyfeldt alone isolated from several cases an organism which he called *B. monocytogenes hominis* (131) Bacteriological examination of the throat has,

on occasions, revealed a high incidence of Vincent's spirochetes but normal controls may, just as frequently, harbor these same organisms (4) Nevertheless, a reasonable number of cases of infectious mononucleosis are associated with an acute Vincent's infection, an occasional one with a beta hemolytic streptococcus tonsillitis, while in the majority of cases an indifferent flora has been cultured from the throat (16)

VI DIAGNOSIS

A General Remarks

As is the case with many diseases, the diagnosis of infectious mononucleosis is frequently overlooked either because the observer is not cognizant of its varying manifestations or because he neglects the consideration of such a diagnosis When a child or young adult is found to have fever and glandular enlargement out of proportion to any local inflammatory condition, an examination of the blood is obviously indicated If, then, there is encountered an increase of the mononuclear elements, including especially the abnormal type of lymphocyte heretofore described, a tentative diagnosis of infectious mononucleosis can be entertained Finally, the benign course of the illness with eventual complete recovery will further substantiate this conclusion Until 1932 some such process epitomized the usual chain of diagnostic events Even when the correct diagnosis was suspected early in the course of the disease, unfamiliar or uncommon phenomena could turn the search into a false channel, from which a return might not be accomplished for days or weeks and only when the obvious recovery of the patient precluded the diagnosis of a more serious malady Not only, therefore, did the Paul-Bunnell test provide a simple laboratory procedure whereby the diagnosis of infectious mononucleosis could be promptly confirmed, but by its application there has been formulated a much more comprehensive conception of infectious mononucleosis as a systemic disease with a variety of manifestations Accordingly we may digress for a brief space to consider the historical background of this test which, although as empirical as the Wassermann reaction in syphilis or the Weil-Felix reaction in the Rickettsial diseases, has aided so materially in advancing the knowledge of infectious mononucleosis

B Heterophile Antibodies—Historical

Heterophile antigens are substances which, when injected into certain animals, will elicit not only specific antibodies but nonspecific antibodies, the presence of these latter being demonstrable by their reaction with antigens other than those involved in their production. One type of these, the Forssman antigen, is a substance which, when injected into rabbits or a group of animals serologically similar, will call forth hemolysins and agglutinins against sheep erythrocytes. Taniguchi (185) in 1920 observed that when guinea pig heart, which contains Forssman antigen, was used as the antigen in the Wassermann test, there was considerable variation in the ability of the unknown sera to fix complement, depending upon the amount of sheep-cell hemolysin contained therein. This variable complement-fixing property could be modified by absorbing out this hemolysin with such a Forssman antigen as guinea pig kidney. Aware of the fact that normal sera might contain fairly large amounts of sheep-cell hemolysin, he concluded that this substance constituted a source of fallacy in the Wassermann reaction when alcoholic extracts of heterogenetic antigens such as guinea pig or horse heart, were employed.

In 1924 Hanganutziu (83), reading the results of routine Wassermann reactions, noted an instance of strong agglutination of the sheep red-cells. This serum, which proved to contain a high titer of sheep-cell antibodies, was found to belong to a patient injected therapeutically ten days before with horse serum. In this case, as well as in other serum treated individuals whom he subsequently studied, he demonstrated increased amounts of agglutinins against red cells of the horse, guinea-pig and several other animals. These antibodies appeared about the tenth or eleventh day after injection and remained for a number of weeks, their presence was independent of the amount of serum administered or the number of injections. In the serum of no individual untreated with horse serum did the normally low titer of hemagglutinins approach the high values seen in serum treated persons. The essential features of these observations were confirmed two years later by Deicher (45), indeed, in Europe, the Paul-Bunnell test is often referred to as the Hanganutziu-Deicher reaction. In an effort to establish a serological test for acute rheumatic fever, because

of its clinical similarities to serum disease in which Davidsohn (37) had found an increase of heterophile antibodies, Paul and Bunnell (136) studied sera from cases of rheumatic fever as well as from a group of hospital patients suffering from other diseases. In the serum of one of this control group, a high titer of sheep-cell agglutinins was demonstrated. This patient had infectious mononucleosis.

C Paul-Bunnell Test

Materials and normal values The materials required to determine the titer of sheep-cell agglutinins are the patient's serum, a suspension of sheep red-cells, and physiological saline solution (the determination of hemolysins requires, in addition, complement). The serum is inactivated for 15 minutes at 56°C, if kept in the icebox, its potency remains constant over a period of several years (16). Starting with 1:4, dilutions of the serum are carried out as far as is indicated. Sheep-cells, collected weekly, are washed three times, from them a 0.67 per cent suspension of packed cells is prepared. To each tube containing 0.5 cc of diluted serum, 1.5 cc of the suspension of sheep cells is added. The tubes are shaken and placed in a 37° water-bath for one hour, then kept overnight in the icebox. The following morning the tubes are gently inverted three times after which any tube in which there is macroscopic agglutination of the sheep-cells is considered positive. The dilution of the serum in the first tube (0.1 cc to a final volume of 2 cc), originally recorded as 1:4, is actually 1:20. Employing the more correct terminology, normal sera may contain sheep-cell agglutinins up to a titer of 1:80. In one series of 300 hospital patients, excluding any with a history of recent horse-serum therapy, the titers of agglutinins were distributed as shown in Table 4 (13). These values were essentially confirmed by several other investigators (41, 177). Females seem to have a slightly higher average titer than males (181).

In infectious mononucleosis For the Paul-Bunnell test to be significantly positive, then, sheep-cell agglutinins must be present to a titer of 1:160 or more. Rarely a normal serum will give a titer of 1:160 or even 1:320 so that this test, like many laboratory procedures, must be interpreted in conjunction with the clinical findings. The highest titers in our 65 cases of infectious mononucleosis were distributed as

indicated in Table 5. A host of authors have corroborated the observation of increased titers of sheep-cell agglutinins in infectious mononucleosis (2, 29, 46, 71, 88, 106, 120, 122, 132, 137, 152, 168, 177, 194, 201). All of these reports have been concerned with sporadic cases, in a single large epidemic (128) positive Paul-Bunnell tests were encountered thus adding a link to the chain of evidence for considering epidemic glandular fever and sporadic infectious mononucleosis different forms of the same disease.

TABLE 4

Distribution of sheep-cell agglutinin titers in 300 hospital patients

	TITER				
	Less than 1:20	1:20	1:40	1:80	1:160
Per cent of cases	29	32	25	14	0

TABLE 5

Distribution of sheep-cell agglutinin titers in 65 cases of infectious mononucleosis

TITER	PER CENT OF CASES	TITER	PER CENT OF CASES
1:20	3	1:2,560	14
1:40	0	1:5,120	11
1:80	5	1:10,240	12
1:160	9	1:20,480	3
1:320	12	1:40,960	0
1:640	11	1:81,920	0
1:1,280	18	1:163,840	2

The titer of sheep-cell agglutinins bears no relation to the severity of the disease or the degree of lymphocytosis. While the usual titer ranges between 1:320 and 1:10,240, values as high as 1:81,920 (21) and 1:163,840 (55) are occasionally recorded. In general, the titers of agglutinins and hemolysins are closely comparable (183), rarely the hemolysins are distinctly lower (25). It will be noted that in our cases, the Paul Bunnell test was positive in 92 per cent, of the five negative results, four were in children 11 years old or younger. While most authors have found the reaction positive in a similarly high percentage of cases (135), Rosenthal and Wenkebach (152) obtained positive

results in only half their cases. If the clinical picture is sufficiently characteristic, therefore, a negative Paul-Bunnell test does not preclude the diagnosis of infectious mononucleosis any more than a negative Wassermann reaction rules out syphilis.

Time of appearance Almost without exception the Paul-Bunnell test is positive, if it is going to become so at all, when first performed, for it is usually not until 4 or 5 days have elapsed that the correct nature of the illness is suspected. In one case the titer of sheep-cell antibodies was 1:5,120 on the third day of the disease, indeed this was the maximum titer attained in that particular instance (16). The test has been found positive on the fourth day (65) and, in a number of our cases, on the fifth or sixth. The peak may be reached by the end of the first week, it is invariably reached by the fourth week. In a case in which sheep-cell antibodies are not increased, the test should be repeated for a month after the onset of illness before efforts to obtain serological confirmation of the diagnosis are relaxed. Sheep-cell agglutinins may be increased several days before any distinctive abnormalities are recognizable in the leucocytes, indeed, in one case with a long prodromal period, an elevated titer was observed 12 days before the abnormal blood count was established (13). Likewise a positive Paul-Bunnell test may antedate glandular enlargement or any of the other clinical features.

Factors influencing titer The agglutinating titer of human sera for sheep erythrocytes is influenced by several factors,—the age of the cells (194, 201), the concentration of cells, the temperature at which the tubes are incubated, and the length of incubation (177). The practical importance of these variable elements is to emphasize the necessity of carrying out the test under closely standardized conditions as otherwise it is impossible to evaluate border-line results. Serum, if kept in the icebox, will show no appreciable change in titer over a period of two years (16).

Duration of antibodies Obviously an important factor determining the duration of increased titers of antibodies is the height of the level attained. The duration varies between wide limits. Davidsohn, for example, observed abnormal titers in 10 cases from 26 to 114 days after the onset of illness with an average period of 56 days (39). An elevated titer may disappear as rapidly as 2 weeks after its appearance. In our cases the earliest return to a normal level occurred

in one within 7 weeks of the onset of illness (from 1 1280 to 1 80) but usually the interval was 4 or 5 months. In 2 cases increased titers existed 5 or 6 months later (16). Thus one may be enabled to diagnose a case of infectious mononucleosis in retrospect long after recovery. This we have done in several instances when a positive Paul-Bunnell test was encountered months after individuals had recovered from a "fever of unknown origin" in which careful scrutiny of hitherto overlooked clinical and hematological records gave support for such a conclusion.

Positive Paul-Bunnell test accidentally discovered in Wassermann laboratory. If, in the course of performing a routine Wassermann test, agglutination of the sheep red-cells is noted, an otherwise unsuspected case of infectious mononucleosis may be uncovered (71).

Modifications, rapid methods. Several modifications of the Paul-Bunnell procedure have been suggested in order to arrive at an earlier conclusion when the diagnosis lies between infectious mononucleosis and conditions requiring immediate specific therapy such as diphtheria, meningitis or appendicitis. A rough method giving an almost immediate result involves the use of a hanging-drop preparation of serum and sheep-cells (29). Employing glass slides similar to those used in the microscopic slide precipitation-test for syphilis, one may obtain equally prompt but more sensitive results (175). A highly delicate technique makes use of smaller amounts of materials and shorter incubation so that results are available within two hours (38), if centrifugation is employed, the test may be read within a few minutes (175).

D Nature of Sheep-Cell Antibodies in Infectious Mononucleosis

Paul and Bunnell assumed that the antibodies occurring in infectious mononucleosis were of the Forssman variety. Very soon, however, there appeared data incompatible with this conception. It was noted that these sheep-cell agglutinins appeared equally regularly in patients with infectious mononucleosis irrespective of the blood group to which they belonged (13, 25, 26). This would be a paradoxical phenomenon since human group A cells contain Forssman antigen. Should Forssman antigen and antibody occur together some sort of reaction would be anticipated. Furthermore the infectious mononucleosis antibodies are poorly absorbed by guinea pig tissues which

efficiently remove Forssman antibodies (177, 176) Continued investigations by Stuart and his collaborators cast doubt on the Forssman-character of the antibodies found in individuals treated with horse serum, since these antibodies are removed to a considerable extent by non-Forssman antigen-containing rabbit red-cells (182) At this point Bailey and Raffel (3), too, arrived by a different approach at the conclusion that infectious mononucleosis antibodies are not of the Forssman type since the hemolysins are absorbed by non-heterophile ox-cells This finding was promptly confirmed (98, 179) and extended to show that in infectious mononucleosis there may be an increase of antibodies against red-cells of sheep, goat, horse or ox but not rabbit, pig, dog and guinea-pig, whereas after injection of horse serum there is an increased titer against the cells of all these species (9) Thus it would seem that there are at least three types of sheep-cell agglutinins,—those in normal serum absorbed by guinea-pig kidney but not by ox-cells, those in infectious mononucleosis serum absorbed by ox-cells but not by guinea-pig kidney, and those in the serum of individuals treated with horse-serum, absorbed by both guinea-pig kidney and ox-cells (178, 183) Further support for the differentiation between the type of antibody response in infectious mononucleosis and serum disease was provided by the observation of high titers of isoagglutinins in the latter condition but normal levels in the former (40) It is sometimes necessary to apply these theoretical principles when, for example an individual, recently injected with horse-serum, is suspected of having infectious mononucleosis, or when a patient is found to have a border-line or normal titer of sheep-cell antibodies To evaluate the significance of the sheep-cell titer under such circumstances Davidsohn has described a differential test for the details of which his papers should be consulted (41, 42) The principles involved he states as follows "The heterophilic antibodies (anti-sheep agglutinin) in infectious mononucleosis are not of the Forssman type They are not absorbed by a suspension of guinea-pig kidney The heterophilic antibodies in serum disease are of the Forssman type and are readily absorbed by a suspension of guinea-pig kidney The anti-sheep agglutinins are promptly absorbed by boiled beef red corpuscles from the sera of patients with infectious mononucleosis and almost as well from the sera of patients with serum disease, but not from normal sera "

E Origin of Antibodies in Infectious Mononucleosis

It is apparent from the above discussion that the sheep-cell antibodies appearing in infectious mononucleosis are in certain respects unique, and cannot be considered to result from a non-specific stimulation of antibodies normally existing in human serum. Indeed, the mechanism of their production is obscure. The source of the antigen which elicits them may be extrinsic or intrinsic. If the former, it must be found in the organism or virus which causes the malady, if the latter, it must be associated with the tissues of the patient, some breakdown product of these tissues or the abnormal leucocytes. The finding of an organism capable of eliciting these antibodies would not necessarily incriminate it as the etiological agent of infectious mononucleosis any more than the positive Widal or Wassermann indicate that the malady is caused by the organisms corresponding to these reactions. Since antibody production is so intimately associated with the spleen, it is pertinent to note that in the instance of an individual whose sheep-cell antibody titer at the time of a splenectomy for thrombocytopenic purpura had been 1:40, the titer during an attack of infectious mononucleosis three years later rose properly to 1:320 (16).

F Relation of Paul-Bunnell Test to Wassermann Reaction

At first glance there might seem to be a direct relationship between the sheep-cell antibodies and the occasionally occurring false positive Wassermann. That this is not the case is indicated by three facts: first, that partial removal of sheep-cell agglutinins makes the complement fixation test more positive rather than decreases its strength (so that any binding effect of these agglutinins upon the sheep red blood cells to prevent hemolysis, is inconsequential), second, that a positive Wassermann may occur in the presence of low titers of sheep cell antibodies while a negative Wassermann may accompany extremely high titers, third, that sheep cell antibodies may persist for many months after the Wassermann test has become negative and may even increase in titer coincident with a reversion of the Wassermann to negative (15).

G Serum Therapy and Infectious Mononucleosis

An injection of horse serum into a patient with infectious mononucleosis does not produce any further increase in sheep-cell antibody

titer beyond that which already exists (9, 122) Serum sickness may appear but the administration of horse serum is fraught with no more danger than in a normal individual (9, 20, 110) Likewise, skin tests with horse serum do not necessarily indicate any abnormal sensitivity (9) One wonders whether skin tests employing sheep-cells might not demonstrate increased sensitivity and thereby serve as a rapid intracutaneous diagnostic procedure for infectious mononucleosis

H Paul-Bunnell Test in Other Conditions

Aside from the increase of sheep-cell antibodies that may be found in horse-serum-treated individuals, the Paul-Bunnell reaction has been found positive only rarely in diseases other than infectious mononucleosis Many thousands of tests have been carried out with negative results in a host of clinical conditions These include the common infectious diseases, the exanthemata, a variety of hematological disorders, syphilis and the other diseases diagnosable by agglutination tests of one sort or another, such maladies associated at times with false-positive Wassermann reactions as yaws and rat-bite fever, and many others having any feature in common with infectious mononucleosis (13, 15) An occasional increased titer has been reported in scarlet fever, rubeola, tuberculosis and filariasis (48) but such events are excessively uncommon Paul and Bunnell (136) found a titer of 1 640 in a young woman suffering either from aleucemic leukemia or aplastic anemia, whose obscure illness terminated fatally This patient had received oral liver therapy which could hardly account for the elevated titer although parenteral liver extract may produce titers as high as 1 1280 (16)

VII PATHOLOGY

A Biopsy

Lymph node Since infectious mononucleosis is such a benign condition, its pathological anatomy has been necessarily inadequately studied What information there is has been gleaned largely from examination of biopsied lymph nodes The most elaborate study was carried out by Downey and Stasney (51), who summarized the pathological changes seen in sections of lymph nodes as follows "Hyperplasia of general and sinus reticulum, nodular foci of rounded reticulum

cells, hyperplasia of follicles and germ centers in one case in which the node was not removed at the peak of the disease, absence of follicles and germ centers in advanced cases, dense areas of small lymphocytes and looser areas in which the lymphocytes vary in size and structure, presence of large lymphocytes with pale nuclei and basophilic lymphocytes with lobulated nuclei in looser areas and in sinuses, more or less obliteration of structure including sinuses depending on degree of involvement, structure never as completely obliterated as in advanced lymphatic leucemia, no invasion of capsule or surrounding tissue by the lymphocytes of the node, presence of groups of plasma cells in most of the nodes "

The pathological picture of the lymph nodes, therefore, is not a uniform one. Indeed the variation in size and shape of the lymphocytes therein contained is usually, as in the peripheral blood, a differential feature from leucemia or, if this characteristic be lacking, the absence of complete loss of structure of the node will ordinarily indicate the benign nature of the process. The same abnormal lymphocytes that appear in the blood stream may be recognized in large numbers in a lymph node (51).

The microscopic appearance of the lymph nodes seems to vary with the stage of the disease at which the biopsy is performed. Accordingly there has been for some years a difference of opinion as to the relative importance of hyperplasia of the lymphoid or the reticulo-endothelial elements. Longcope (109) emphasized the hyperplasia of both types, the occasional presence of large epithelioid cells and, in one case, found the picture indistinguishable from Hodgkin's disease. Pratt (143) studied two nodes removed from himself, one during the acute stage of the illness, the second a year later. The first of these showed hyperplasia, early degenerative changes in the vessel walls, hemorrhages, and such marked reticulo-endothelial proliferation, with some central necrosis, as to exclude almost all lymphoid elements. The second node which was somewhat fibrotic, still showed considerable reticulo-endothelial hyperplasia. Hyperplasia of the reticulum was stressed by Gooding (74) and Davidsohn (38). Since lymphocytes may develop from reticulum this discussion assumes largely an academic air, furthermore different areas of the same lymph node may give support for both the reticulo-endothelial and lymphatic

schools of thought (50) The presence exclusively of lymphoid hyperplasia, has, nevertheless, been emphasized by a number of observers (4, 33, 49, 113, 154, 174)

Tonsil, bone-marrow Histological examination of a tonsil revealed marked proliferation of small mononuclear cells (62) In the bone-marrow, as in the lymph nodes, there may be large numbers of the atypical lymphocytes seen in the blood (52, 63, 207)

B Autopsy

Since the few fatalities recorded have followed secondary infection, usually a septicemia, the autopsies are of little value in the present discussion (77, 81, 189) It is perhaps worthy of mention that in DuBois' (52) case, in which death followed an empyema, the spleen showed atrophy of the Malpighian corpuscles, endothelial proliferation of the sinuses and distension of lymph spaces by mononuclear cells, many with mitotic nuclei The portal spaces of the liver were infiltrated by large mononuclear cells What changes there were in the lymphoid elements, he regarded as secondary to oedema of the primarily-involved reticulo-endothelial tissues

VIII DIFFERENTIAL DIAGNOSIS

By virtue of its protean manifestations, infectious mononucleosis has one or several features in common with a host of other diseases It is so fundamentally different from many of these that no further discussion is required than to mention that there exists a superficial similarity the significance of which vanishes upon more careful detailed observation However so closely does it simulate certain conditions that immediate differentiation even with the aid of elaborate methods may be impossible and a protracted period of observation be required before a final conclusion can be reached The Paul-Bunnell test is always applicable when such problems arise

A Hematological Disorders

Leucemia In atypical cases, the distinction from leucemia may be difficult, indeed an occasional so-called transitional form between the two diseases has been observed (167) In acute lymphatic or monocytic leucemia hemorrhagic phenomena and anemia are common, a

uniform type of immature cell is encountered, in infectious mononucleosis bleeding and anemia are rare, the type of cell is older and variable in its characteristics (91) A bone-marrow biopsy may be inconclusive (63) as may the microscopic appearance of a lymph node removed early in the illness, but in a well-established chronic lymphatic leucemia the characteristic features of an invasive process are present (51) It is in the differentiation from leucemia that the Paul-Bunnell test is most valuable, for in all types of leucemia not only is the sheep-cell titer not elevated but it is uniformly confined to abnormally low levels, below 1:20 (14, 40, 122, 200)

Leucopenic infectious monocyto-sis This disease typically affects older individuals more than does infectious mononucleosis It is characterized by necrotizing lesions of the oral cavity, marked leucopenia and absence of lymphadenopathy or splenomegaly (150, 151)

Agranulocytosis Agranulocytosis is more common in older females, infectious mononucleosis in younger males (169) In the former there is usually a history of drug ingestion Although there may be leucopenia with a profound depression of the myeloid elements in infectious mononucleosis, the atypical lymphocytes ordinarily aid in arriving at a correct diagnosis Nevertheless these abnormal cells do occasionally appear in agranulocytosis so that at the onset the two conditions may be readily confused (51) Such has not infrequently happened (39, 54) with the result that pentnucleotide has been administered unnecessarily in instances of infectious mononucleosis (13, 84)

Thrombocytopenic purpura It is only necessary to recall that infectious mononucleosis may closely mimic purpura hemorrhagica with hemorrhagic phenomena, prolonged bleeding time, reduced platelets and a positive tourniquet test so that one is in doubt whether he is dealing with one or the other of these two diseases or even both together (13, 123, 124, 187)

B Diseases Associated with Sore Throat

Vincent's angina In Vincent's angina there may be a lymphocytosis as high as 70 or 80 per cent (66, 203), conversely a Vincent's infection may be the presenting clinical feature of infectious mononucleosis (84) Certain but not all cases of Vincent's angina with lymphocytosis undoubtedly represent instances of infectious mono-

schools of thought (50) The presence exclusively of lymphoid hyperplasia, has, nevertheless, been emphasized by a number of observers (4, 33, 49, 113, 154, 174)

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Since the few fatalities recorded have followed secondary infection, usually a septicemia, the autopsies are of little value in the present discussion (77, 81, 189) It is perhaps worthy of mention that in DuBois' (52) case, in which death followed an empyema, the spleen showed atrophy of the Malpighian corpuscles, endothelial proliferation of the sinuses and distension of lymph spaces by mononuclear cells, many with mitotic nuclei The portal spaces of the liver were infiltrated by large mononuclear cells What changes there were in the lymphoid elements, he regarded as secondary to oedema of the primarily-involved reticulo-endothelial tissues

VIII DIFFERENTIAL DIAGNOSIS

By virtue of its protean manifestations, infectious mononucleosis has one or several features in common with a host of other diseases It is so fundamentally different from many of these that no further discussion is required than to mention that there exists a superficial similarity the significance of which vanishes upon more careful detailed observation However so closely does it simulate certain conditions that immediate differentiation even with the aid of elaborate methods may be impossible and a protracted period of observation be required before a final conclusion can be reached The Paul-Bunnell test is always applicable when such problems arise

A Hematological Disorders

Leucemia In atypical cases, the distinction from leucemia may be difficult, indeed an occasional so-called transitional form between the two diseases has been observed (167) In acute lymphatic or monocytic leucemia hemorrhagic phenomena and anemia are common, a

uniform type of immature cell is encountered, in infectious mononucleosis bleeding and anemia are rare, the type of cell is older and variable in its characteristics (91) A bone-marrow biopsy may be inconclusive (63) as may the microscopic appearance of a lymph node removed early in the illness, but in a well established chronic lymphatic leucemia the characteristic features of an invasive process are present (51) It is in the differentiation from leucemia that the Paul-Bunnell test is most valuable, for in all types of leucemia not only is the sheep-cell titer not elevated but it is uniformly confined to abnormally low levels, below 1:20 (14, 40, 122, 200)

Leucopenic infectious monocytois This disease typically affects older individuals more than does infectious mononucleosis It is characterized by necrotizing lesions of the oral cavity, marked leucopenia and absence of lymphadenopathy or splenomegaly (150, 151)

Agranulocytosis Agranulocytosis is more common in older females, infectious mononucleosis in younger males (169) In the former there is usually a history of drug ingestion Although there may be leucopenia with a profound depression of the myeloid elements in infectious mononucleosis, the atypical lymphocytes ordinarily aid in arriving at a correct diagnosis Nevertheless these abnormal cells do occasionally appear in agranulocytosis so that at the onset the two conditions may be readily confused (51) Such has not infrequently happened (39, 54) with the result that pentnucleotide has been administered unnecessarily in instances of infectious mononucleosis (13, 84)

Thrombocytopenic purpura It is only necessary to recall that infectious mononucleosis may closely mimic purpura hemorrhagica with hemorrhagic phenomena, prolonged bleeding time, reduced platelets and a positive tourniquet test so that one is in doubt whether he is dealing with one or the other of these two diseases or even both together (13, 123, 124, 187)

B Diseases Associated with Sore Throat

Vincent's angina In Vincent's angina there may be a lymphocytosis as high as 70 or 80 per cent (66, 203), conversely a Vincent's infection may be the presenting clinical feature of infectious mononucleosis (84) Certain but not all cases of Vincent's angina with lymphocytosis undoubtedly represent instances of infectious mono-

nucleosis, differentiation can be effected only through the application of the Paul-Bunnell test (65)

Diphtheria Certain supposed cases of diphtheria with negative cultures have, after antitoxin therapy, been recognized as examples of infectious mononucleosis (16, 20, 33, 47, 148) Examination of the blood and bacteriological studies should minimize the occurrence of such errors

Follicular tonsillitis A typical B-hemolytic streptococcic follicular tonsillitis may usher in an attack of infectious mononucleosis (16) A simple tonsillitis is generally of shorter duration than infectious mononucleosis (153)

Herpetic pharyngitis or aphthous stomatitis Here no changes in the blood are recognized (108) An aphthous stomatitis may, however, be the most prominent sign of a case of infectious mononucleosis (70)

C Diseases Associated with Glandular Enlargement

Syphilis Infectious mononucleosis may occur in individuals with early (53, 60) or late syphilis (4) Secondary syphilis may be closely simulated by infectious mononucleosis (33, 149), when a false-positive Wassermann is present, there is introduced further confusion which a blood smear and the Paul-Bunnell test usually dispels

Hodgkin's disease The more insidious onset of Hodgkin's disease will usually indicate the proper diagnosis

Tuberculosis Instances of disseminated tuberculosis with a lymphocytosis as high as 87 per cent (44) or 97 per cent (102) have been reported

Tularemia The glandular enlargement of tularemia in conjunction with lesions in the throat may be confusing, especially since there may be a lymphocytosis as high as 79 per cent (16) No instance of false-positive agglutination of B tularensis in infectious mononucleosis has been reported, however

Pertussis The thoracic form of infectious mononucleosis may be mistaken for pertussis particularly since, in the latter, the lymphocytes may be increased up to 94 per cent (44)

Granuloma inguinale This must be distinguished from the inguinal form of infectious mononucleosis (33)

Mossman river fever This supposedly insect-borne infection occurs in Australia, affects adults, is characterized by glandular enlargement especially of the axillary and inguinal groups, a macular eruption, a benign course and a tendency to recur at yearly intervals. There is a leucocytosis as high as 13,000 with lymphocytosis up to 45 per cent (24).

Haberfelds' disease The Habersfelds encountered in Brazil a presumably tick-borne infection, prevalent in the spring and fall, manifesting itself by general glandular enlargement, usually most marked in the axillae, and a normal leucocyte count with over 60 per cent lymphocytes. Victims seem to develop a permanent immunity (80).

Dengue In dengue there is typically a skin rash, glandular enlargement chiefly inguinal, leucopenia with relative lymphocytosis up to 60 per cent. The spleen is rarely enlarged (79, 85).

Mumps The earlier writers were much concerned by the confusion between these two diseases. They emphasized, as have more recent observers, the similar onset with unilateral cervical swelling progressing to contralateral involvement (32, 61, 69, 202, 204). Occasionally in mumps there may be a relative mononucleosis as high as 76 per cent (198), associated with infectious mononucleosis there may be an orchitis (134). A previous history of mumps is of assistance.

Thyroiditis Epidemic thyroiditis which occurs in South America, is caused by a trypanosome, and is characterized by general glandular enlargement as well as thyroid enlargement (4).

D Diseases Associated with Cutaneous Eruptions

Chickenpox Lymphocytosis in varicella is not uncommon although it rarely attains the values reported by Goldman (72) in a three year old child—81,200 leucocytes with 89 per cent lymphocytes. We recently saw a medical student suffering from varicella who had general glandular enlargement, 8,000 leucocytes of which 48 per cent were mononuclear cells (a number of these were the typical abnormal lymphocytes seen in infectious mononucleosis), but a negative Paul Bunnell test.

Scarlet fever A scarlatiniform rash (9) which may even be followed by desquamation (145) can lead to an erroneous diagnosis of scarlet fever.

German measles Parkes Weber has emphasized the "lymphotropic" blood picture common both to German measles and infectious mononucleosis, which may, in the presence of a rash, introduce confusion (197, 198)

Erythema nodosum and multiforme It is only necessary to mention that eruptions of both types may occur in infectious mononucleosis

Influenza Probably instances of infectious mononucleosis pass unrecognized more frequently under the mistaken diagnosis of influenza than of any other condition. Many of these errors could be rectified by searching for glandular enlargement and examining a blood smear, the rest by carrying out the serological test in questionable cases

E Miscellaneous Infections

Typhoid fever Infectious mononucleosis and typhoid fever may resemble one another in a variety of features including headache, epistaxis, bradycardia with a dicrotic pulse, splenomegaly, rose-spots, leucopenia and lymphocytosis (which in typhoid fever may reach 80 per cent (52)). Consequently the distinction between the two diseases may be extremely difficult (53). When one recalls, further, that the Widal may be positive in infectious mononucleosis (13, 121), and, finally, that the two conditions may occur together (163), it is obvious that differentiation may, at times, have to wait upon the clinical course. Any person assumed to be suffering from typhoid fever who does not provide cultural confirmation of this diagnosis should be carefully examined for evidence of infectious mononucleosis.

Undulant fever The onset of infectious mononucleosis and undulant fever may be very similar, a false-positive agglutination for *B. melitensis* in the former may be correspondingly misleading.

Malaria Since infectious mononucleosis and malaria may occur simultaneously, the respective signs may be difficult to unravel (115). Furthermore there may be a superficial similarity in the blood smear (52).

Acute rheumatic fever There are certain obvious features including cardiac involvement which infectious mononucleosis and acute rheumatic fever have in common. A more careful analysis should permit ready differentiation.

Bacterial endocarditis In bacterial endocarditis a monocytosis as high as 80 per cent (52) might offer a temporary barrier to correct interpretation of fever, splenomegaly and hematuria

Pneumonia The focussing of attention on the pulmonary manifestations with a disregard of other signs could delay a proper diagnosis of infectious mononucleosis

Acute infections of the central nervous system When one recalls that in infectious mononucleosis there may be symptoms and signs of meningeal irritation as well as abnormalities in the cerebrospinal fluid, it is obvious that immediate differentiation from pyogenic or benign lymphocytic meningitis, encephalitis or poliomyelitis may be impossible. Even in retrospect it is impossible to be certain about the nature of the following case. A young man developed fever, signs of meningeal irritation, general glandular enlargement and a palpable spleen. The leucocyte count was 8,100, polymorphonuclears 51 per cent, large lymphocytes 2 per cent, small lymphocytes 39 per cent, atypical lymphocytes 8 per cent. The Paul-Bunnell test was negative, recovery was complete (16)

Acute abdominal conditions In any young person with an acute abdominal condition presenting unusual signs, a blood smear should be examined and, if indicated, a Paul-Bunnell test performed so that an unnecessary laparotomy may be averted (38, 74)

Catarrhal jaundice The ultimate differentiation between catarrhal jaundice and infectious mononucleosis with jaundice may rest entirely upon the Paul Bunnell test as in the following case. A young physician with characteristic signs of catarrhal jaundice had a leucocyte count of 3,900, polymorphonuclears 38 per cent, large lymphocytes 26 per cent, small lymphocytes 30 per cent, monocytes 6 per cent. The Paul Bunnell test was negative (16)

F Other Diseases

Nephritis In instances of atypical acute nephritis, particularly where hematuria is out of proportion to the other signs, infectious mononucleosis must be eliminated as the basis of the renal manifestations

Serum disease In addition to the obvious clinical similarities between infectious mononucleosis and serum disease including fever,

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Serum disease In addition to the obvious clinical similarities between infectious mononucleosis and serum disease including fever,

rashes, glandular enlargement and joint pains, further confusion is introduced by the lymphocytosis (11) and false-positive Wassermann reaction that may accompany serum sickness (16, 65, 106) When doubt concerning the validity of the original diagnosis arises in an individual who has received an injection of horse-serum and is later suspected of having infectious mononucleosis, it is necessary to apply differential absorption tests (9, 41)

Drug therapy The administration of adrenalin (111) and quinine (191) is recognized as a cause of transitory lymphocytosis

IX ETIOLOGY

A Pathogenesis

In general, the earliest authors considered infectious mononucleosis an infectious process Certain of them, however, struck by the frequency of constipation as a symptom, made the logical but improbable suggestion that the disease started as a functional disturbance of the gastro-intestinal tract Thence they postulated a spread through lymphatic channels to the lymph-nodes of the neck, a scheme which was in accordance with the initial enlargement of the left cervical nodes (77, 87)

As the more varied manifestations of infectious mononucleosis were brought to light, it became apparent that the disease was probably a generalized infection whose many possible presenting features, whether sore throat, meningitis or jaundice, were secondary to a primary systemic disorder (56) Irrespective of whether the lymphoid (113) or the reticulo-endothelial (52) tissues were considered to be involved initially, the balance of opinion came to favor the view that some infectious agent localized in the lymph nodes (51) with subsequent enlargement of these structures representing a protective reaction of the body against this organism Parkes Weber (187) further conjectured that "all the symptoms of the disease were manifestations of protective reaction, excepting those that were due to the direct action of the invaders and their toxic products"

B Possible Relations with Other Diseases

Leucemia The cause of infectious mononucleosis is unknown So it is that many theories have been formulated, the majority based on

circumstantial evidence with little experimental support. For example the hypothesis has been suggested that infectious mononucleosis is the initial stage of a disease which may terminate in leucemia (70). A single case offered in defense of this view is hardly convincing since the argument is based largely upon a similarity in microscopic appearance of the bone marrow in an instance of infectious mononucleosis and in one of acute lymphatic leucemia (63).

Vincent's infections The association between infectious mononucleosis and Vincent's invasion of the oral cavity is so frequent as to seem more than a fortuitous occurrence (19, 84, 159). Nevertheless there is little proof that this coincidence indicates anything more than that the circumstances which predispose an individual to contract infectious mononucleosis may cause him likewise to be susceptible to a Vincent's infection, since the Vincent's infection may precede, appear simultaneously with or follow the infectious mononucleosis (16). Infectious mononucleosis has developed in individuals under active arsenical therapy for a pre-existing syphilis (4, 203), a fact which has been taken to indicate that the Vincent's organisms could not be the cause of infectious mononucleosis (84). This argument is not convincing, however, for most of these same patients showed manifestations of Vincent's angina, a process which should likewise have been prevented by the antecedent treatment if this reasoning were valid. Guinea-pigs inoculated with portions of a membrane removed from the throat of a young girl suffering from Vincent's angina and infectious mononucleosis, developed a typical blood picture of infectious mononucleosis as did other guinea pigs injected with living or dead strains of a vibrio isolated from cases of Vincent's infections (76). This experimental work has never been confirmed so that there is really no sound basis for considering Vincent's organisms to be the etiological agents in infectious mononucleosis.

Syphilis, rubeola Infectious mononucleosis may develop coincident with early syphilis (60), this, too, is undoubtedly by chance. Glanzmann, impressed with the clinical similarities between infectious mononucleosis and rubeola, concluded that the diseases must be caused by closely related lymphotropic viruses (70).

Influenza It is of interest that while the name of Emil Pfeiffer is so intimately bound up with the early history of infectious mono-

nucleosis, that of R. Pfeiffer is connected with the discovery of the influenza bacillus. An early author (77) concluded that infectious mononucleosis represented the lymphatic form of influenza, a notion repeatedly expressed as late as 1935 in a discussion of infectious mononucleosis entitled "Influenza Lymphatica" (129). One epidemic of infectious mononucleosis in children was immediately preceded by an outbreak of adult influenza (128). Likewise in the course of an epidemic of influenza in Baltimore adults in 1931, a number of children were seen with fairly typical infectious mononucleosis (94). On the other hand infectious mononucleosis was certainly not recognized if it was prevalent during the influenza pandemic of 1918-19 (189). Nor is there any reason to associate the causative agent of infectious mononucleosis with that of rheumatic fever beyond the occasional instance of the former disease followed by a cardiac valvular lesion (22).

C Animal Inoculation Experiments

Monkeys On the whole, cultures from the blood and lymph nodes have yielded disappointing results. With rare exceptions animal inoculations have been equally fruitless. Thus negative results followed the injection of material from a lymph node into guinea-pigs (113), rabbits (4), mice and monkeys (58). Very recently Wising (205), by injecting emulsified material from lymph nodes of several patients with infectious mononucleosis, produced in *Macacus* monkeys a mild febrile illness with general glandular enlargement, normal leucocyte count but a slight mononuclear increase. This disease was transmissible from monkey to monkey, a laboratory worker likewise contracted infectious mononucleosis by accidentally piercing his finger with a knife which had been in contact with a gland removed from one of the infected animals. The Paul-Bunnell test was not carried out on the monkeys, in the human experimentally elicited case, the test was positive.

Toxoplasma Bland (17, 18) isolated from the blood of one out of five cases of infectious mononucleosis a protozoon of the genus *Toxoplasma*, which he named the GF I strain. The experimental disease which he produced in rabbits by the injection of blood from this human case was transmissible serially to other rabbits. It was characterized by fever, rapidly progressive anemia, leucopenia with an absolute

monocytosis, enlargement of lymph nodes, spleen and liver, these organs containing focal necroses. Furthermore, and a feature which differentiates this from other known types of toxoplasmata, the GT I strain injected into monkeys produced fairly regularly a febrile illness with anemia, lymphocytosis, glandular enlargement and splenomegaly. A rabbit inoculated with the monkey's blood contracted, in turn, a typical attack of the experimental glandular fever and protozoa were found in its organs. The pathological picture in the monkey differed from that in the rabbit in the absence of focal necroses, nor were the protozoa demonstrable at autopsy in the organs of the monkey. In rabbits the disease is fatal, in monkeys it is benign. These experiments were repeated by Minkenhof (122) but gave negative results, and have, moreover, never been confirmed by anyone else.

B. monocytogenes hominis. From the blood of several human cases of infectious mononucleosis, Nyfeldt cultivated a small, slowly growing Gram positive bacillus which he named *B. monocytogenes hominis* (131). Injected into dogs this organism elicited a mononucleosis. Anton (1) confirmed the essential features of this work, noting that the canine disease, which was contagious, had many similarities to infectious mononucleosis in man. More recently Schmidt and Nyfeldt (160) grew this same organism from the cerebrospinal fluid of 4 out of 5 patients with infectious mononucleosis, in only one of whom was there clinical evidence of meningitis. Nyfeldt's bacillus belongs to the genus *Listerella* which occasionally produces meningitis in man. Other members of this group have brought about spontaneous epidemics in rabbits and guinea pigs of a disease associated with mononucleosis but otherwise, neither in the animal or human varieties of *Listerella* infections, is there any resemblance to infectious mononucleosis (196).

D. Miscellaneous Organisms

There is no convincing proof that the streptococcus is the cause of infectious mononucleosis although this organism has occasionally been cultured from lymph nodes (77, 189). A diphtheroid of dubious significance was isolated from a single case (183). *B. fecalis* alkaligenes has been suggested as the etiological agent (145). Mazet (116) demonstrated in blood smears from a patient with infectious mono-

this material might well prove to be an invaluable agent for the purposes of stimulating a broad non-specific protective response against infections in general

Finally, a plea may be made for a wider routine performance of the Paul-Bunnell test, not only as a confirmatory procedure in infectious mononucleosis or as one of the diagnostic agglutination tests carried out in instances of "fever of unknown origin," but likewise under the following circumstances

- 1 Individuals with an unexplained lymphocytosis or glandular enlargement
 - 2 Individuals with positive agglutination tests (Widal, etc) without cultural confirmation
 - 3 Individuals with false-positive Wassermann reactions
 - 4 Individuals with unexplained acute abdominal conditions
 - 5 Individuals with enlarged spleens
 - 6 Patients with atypical forms of conjunctivitis or puffy eyelids
 - 7 Apparent cases of (a) catarrhal jaundice, (b) purpura hemorrhagica, (c) Vincent's infections, (d) aphthous stomatitis, (e) benign lymphocytic meningitis, (f) agranulocytosis
- By adopting such an attitude of suspicion, further manifestations of infectious mononucleosis will be unearthed and additional clues be disclosed pointing toward the etiology of this interesting condition

XII SUMMARY

Infectious mononucleosis, glandular fever, monocytic angina, and lymphoid-cell angina are probably different manifestations of the same disease the etiology of which is unknown. Its commonest clinical features are sore throat, fever and lymph node enlargement but none of these are essential signs. Infectious mononucleosis may be associated with abdominal pain, puffiness of the eyelids, conjunctivitis, pulmonary signs, cardiac involvement, jaundice, nephritis, hemorrhagic phenomena, a variety of cutaneous eruptions and central nervous system changes. The mortality in uncomplicated cases is nil, no serious sequelae are recognized. Diagnosis can be confirmed by characteristic changes in the leucocytes and established by the Paul-Bunnell test which is highly specific. There may be encountered a number of confusing serological reactions including a false-positive Wassermann

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THE URIC ACID CONTENT IN BLOOD AND URINE IN HEALTH AND DISEASE

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INTRODUCTION

Uric acid was discovered in 1776 by Scheele (370) and Bergman (25), simultaneously, and in the following years a number of more or less well founded theories were advanced as regards the connection between disturbances in the uric acid metabolism and various diseases, particularly gout. In 1797 Wollaston (443) found uric acid in gouty deposits, and about the middle of the nineteenth century Garrod (140) demonstrated an accumulation of uric acid in the blood

of patients with gout A large number of investigators made determinations of the uric acid excreted by patients suffering from the most widely varying diseases and, gradually, uric acid came to play a prominent part in the medical world, a large number of diseases being included under the heading uric acid diathesis at about the close of the nineteenth century

However, the more exact methods of physiologico-chemical investigation available in modern times have not made it possible to provide support for the maintenance of this clinical theory even within somewhat narrow limitations

Although there may possibly be certain pathogenetic features common to the diseases included in this diathesis, a disturbance of the purine metabolism can hardly be admitted Anomalies in the metabolism of uric acid that may be assumed to be of a certain pathogenetic importance seem to appear only in typical gout, although it has not been possible, in spite of the most extensive investigations, fully to define their nature

The determination of the content of uric acid in blood and in urine has, however, maintained its place in clinical medicine, principally in the diagnosis of gout, though also, with more or less justification, in other fields Among these, uric acid determination has of recent years been considered important to the early diagnosis of absolute renal insufficiency, which, again, has made the question of the renal excretion of uric acid to a certain extent a vital one

METHODS OF ANALYSIS

During the course of time a very large number of methods for the quantitative analysis of uric acid have been published The methods employed clinically for the determination of uric acid in smaller quantities of blood and of urine have, however, been constantly handicapped by some uncertainty—especially in the case of blood analyses This is shown by the frequent appearance of proposals for new methods, or modifications of older ones A common feature of by far the majority of the methods employed is the isolation of the uric acid in the samples as a salt of low solubility, followed by the attempted determination, by various methods, of the quantity isolated

Originally the amount isolated was weighed, after it had been dried

and purified. This method, which required the employment of large quantities, was especially applicable to the examination of urine.

However, as early as the middle of the last century Garrod (140) succeeded in elaborating his famous thread test for the demonstration of uric acid in small quantities of blood. After the addition of acetic acid to blood serum the mixture is allowed to stand for some days, when uric acid crystals are visible microscopically on a couple of fine threads. The method allowed quantitative evaluation but was only applicable in cases of hyperuricemia.

Efforts were gradually made, however, to elaborate practical quantitative methods of analysis on the basis of more exact chemical reactions, and an exceedingly large number of these methods have been evolved during the last fifty years. The most important of them will be briefly reviewed in the following section.

The phosphotungstic acid method

The methods in general use at present for the analysis of uric acid in blood and in urine are based on the observation that uric acid in alkaline solution gives an intense blue with phosphotungstic acid.

This reaction has been known since the close of the last century (125, 300, 318, 347) but was not used for quantitative analysis until 1912 [simultaneously by Folin and Macallum (118) and Riegler (348)].

The original methods have since been subjected to many modifications, of which those elaborated by Benedict (15) in 1922 and by Folin (108) in 1933 have been employed most extensively.

It very soon appeared that the reaction was by no means specific for uric acid but that a great many different substances reacted in the same way, though to different degrees. The principal of these are ergothionein, glutathion, ascorbic acid, and phenols, to which must be added various metals (Mg, Zn, Cu, Fe, Ag, Ni) and other inorganic reducing agents [H_2S , $(\text{NH}_4)_2\text{S}$]. Various proteins, aldehydes, hexoses, pentoses, various alcohols, hemoglobin, hematin, chlorophyll, urinary pigments, etc., etc., also react (17, 22, 48, 76, 117, 197, 245, 371).

On the other hand, it has been found that certain substances, especially amino acids, inhibit the reaction between uric acid and phosphotungstic acid (68, 69, 109).

The reaction between many of the substances mentioned and the uric acid reagent is due presumably to the fact that most of the tungsten preparations also contain molybdenum, which is indicated by greater or lesser blind values obtained by the use of many of the preparations (34, 48, 107, 108, 109, 119, 122, 316, 325, 424)

Intensive research has therefore been directed, especially at Folin's laboratory, towards the production of methods for the preparation of quite pure phosphotungstic acid reagents, while others (15, 200, 201, 217, 305, 316) have tried to find tungstic compounds that react more specifically—such as arseno-18-tungstic acid and arseno-phosphotungstic acid—though it cannot be said that the results have been entirely successful (34)

On account of the non-specificity of the reaction, attempts have been made from the onset to isolate uric acid from the samples as a salt of low solubility by trying to precipitate the uric acid as completely as possible and then to re-dissolve it quantitatively in a solvent that does not disturb the subsequent course of the reaction

The reagent most often used is silver lactate, sometimes in combination with magnesium sulphate (20, 69, 107, 108, 112, 362, 363, 402) Others prefer precipitation with salts of zinc, nickel, or mercury, after which the final solution is considered to be clearer (79, 176, 217, 301, 305) Uric acid is often isolated in urine analyses as ammonium urate (194, 348)

Various dilutions of strong acids were originally used as solvents (112), a solution of 10 per cent NaCl in N/10 HCl (123) having generally been employed in recent studies Benedict and Hitchcock (20) introduced 5 per cent sodium cyanide as a solvent, which renders further addition of alkali superfluous and accentuates the final blue color The eventual intensity of the color is further increased, and the solution is clearer, when urea is added to the cyanide solution (107)

While it has been comparatively easy to isolate the uric acid in urine, the difficulties have been considerably greater as regards the small quantities of uric acid in blood and in serum, which are furthermore diluted by the previous protein precipitation Some authors state that they can recover a definite addition of uric acid almost quantitatively, others lose from 15 to 30 per cent, or more, of the quantity added (34, 39, 69, 178, 202, 325, 354)

The problem is greatly complicated by the fact that ions dissolved from the precipitating reagents and the impurities in the tungstic acid reagents may apparently affect the final color-reaction both positively and negatively (108, 217, 305, 325, 440). This is, presumably, why the values found by indirect (after isolation) analysis are not infrequently higher than those found by direct (without isolation) analysis (325). The quantity of uric acid added could not be recovered in a large number of cases, even by direct analysis. "Uric acid added to blood has never been quantitatively recovered by the direct method, when applied to the filtrates from laked blood" (Folin 1933) (108). That Folin and others have thought that they had recovered quantitatively an added amount of uric acid in a number of serum or unlaked blood tests is presumably due to the fact that the blind value, which is independent of the amount of filtrate, has substituted the quantity of uric acid lost when suitably less filtrate than usual was employed after the addition of uric acid.

Many authors emphasize that the reaction of uric acid with the uric acid reagent is not stoichiometric, as they only found proportionality between the concentration and the intensity of the color within a very restricted range (19, 34, 85, 108, 214), others found agreement with Beer's law within wide limits (181, 359). The differences between the results obtained by various authors may be considered to be due to differences in unknown details of the reagents employed.

The chemical reaction between uric acid and the uric acid reagent is not known exactly, but it is undoubtedly very complex. Folin (109) emphasizes in his last study, published in 1934

"probably less than 10 per cent of the color obtained in the modern forms of the method represents the direct reduction of the reagent by the urate. The other 90 per cent (more or less) represents indirect or induced reduction due to the presence of the cyanide. The latter reduction is rather more specific for uric acid than the directly produced smaller fraction. The indirect reduction is subject to great variation in magnitude according to the quality of the uric acid reagent, the quality of the cyanide, and other modifying factors, such as the degree of alkalinity, the presence of carbonate, of amino compounds, of some phenol products etc."

Fairly considerable variations in the intensity of the color can be found not only with reagents of different ages but also with the same reagent, and the blind value found by many authors is also very variable (34, 48, 316, 325, 424) Finally, a certain relation exists between the concentration of uric acid and the speed of the reaction (34, 214)

Various authors have tried to use photometry as an improvement on the colorimetric methods of analysis (34, 103, 181, 214, 217, 232) The frequent turbidity of the solution will, however, cause still more trouble in the photometric than in the colorimetric readings (34)

Finally, an attempt has been made to replace colorimetric readings by titrating to decoloration with potassium ferricyanide (209, 210, 360), chloric water, or sodium hypochlorite (427, 428) But the main source of error, defective specificity and stoichiometry, is not thus avoided

The potassium permanganate method

Hopkins (193) evolved a method in 1892 by which the uric acid in the urine was isolated as ammonium urate, dissolved in hydrochloric acid, and titrated with potassium permanganate This method was modified by several investigators (73, 120, 304, 362, 363, 373, 445, 446) who altered, especially, the method of isolation Many of these modifications, particularly Salkowski and Ludwig's (363) and Folin and Shaffer's (120), have proved to be quite suitable for urine analyses They all, however, take much time and need large samples No practical methods based on similar principles have been evolved for blood analyses (302, 303)

The iodine method

Uric acid in alkaline solution reacts with iodine, but the reaction is dependent on the acidity, the time, the presence of various salts, etc (47, 133, 134, 158, 198, 229, 299, 426) A number of investigators have tried to utilize this principle for the analysis of uric acid after isolation (84, 134, 137, 158, 229, 342, 356) However, these methods have, apparently, not found any extensive application outside France Curtman and Lehrman (80), working on similar principles, evolved a rather complicated method of analysing the content of uric acid in blood after isolation as nickel urate

The reaction of uric acid with iodine seems, however, to be subject to the influence of too many only partially known factors to be of any practical clinical use at present

The potassium ferricyanide method

Schlieper (378) demonstrated as early as 1848 that uric acid reduces potassium ferricyanide. Uric acid in neutral or alkaline solution is oxidized to allantoin. The reaction takes place fairly quickly even at a low temperature and in considerable dilution.

Flatow (100) stated in 1925 that he had found it to be a stoichiometric reaction, one molecule of uric acid constantly reducing two molecules of potassium ferricyanide. In the following years he elaborated on this basis methods for the determination of uric acid in blood and in urine (101, 102). For serum analysis protein precipitation was carried out with uranyl acetate. A definite excess of potassium ferricyanide was added to a certain quantity of filtrate and, after standing at room temperature for two minutes, the potassium ferricyanide remaining was titrated with a solution of monosulphindigotic sodium, two molecules of potassium ferricyanide oxidizing one molecule of the indigo compound. In cases of analysis of total blood or urine the uric acid was isolated in accordance with the usual principles.

Without giving any further account of the results of his experiments, Flatow states that the method is very accurate, the error being only 0.001 mg. (for the analysis of from 0.3 to 0.5 cc. serum).

Blankenstein (28), however, only recovered from 31 to 64 per cent of an added quantity of uric acid when the protein precipitation was carried out with uranyl acetate, and from 72 to 100 per cent after precipitation with tungstate.

Various authors (8, 9, 34, 293, 355, 398, 418) evolved more or less important modifications of the method during the succeeding years, the less practical titration with the indigo compound having been replaced by iodometric titration, or by colorimetric or electrometric measurement.

Though the ratio given by Flatow, that one molecule of uric acid constantly reduces two molecules of potassium ferricyanide, was generally accepted, some authors (34, 398) found that the ratio between the quantity of uric acid and that of potassium ferricyanide

was not constant for the amounts usually employed in the analysis, others found that a certain amount of uric acid reduced various quantities of potassium ferricyanide in various experimental conditions (132, 166)

More exact investigation has shown that the reaction between uric acid and potassium ferricyanide is dependent on a number of different factors (34)

Uric acid reduces no measurable quantities of potassium ferricyanide at, and below, pH 3, increasing quantities with a rising pH, and a maximal and constant quantity at pH 10–14. The reaction is in some peculiar way dependent on time when pure uric acid solutions are examined. The quantity of potassium ferricyanide reduced reaches its maximum at 20°C after two minutes, when one molecule of uric acid at pH 11 generally reduces three molecules of potassium ferricyanide [i.e. 1 mg uric acid reduces 3.57 cc N/200 $K_3Fe(CN)_6$]. The quantity of ferricyanide reduced decreases when the mixture is allowed to stand for a longer period, until, after from 15 to 30 minutes, it is found that 1 mg reduces 2.90 cc N/200 $K_3Fe(CN)_6$. The course of the reaction is seen to be the same at higher and lower temperatures, though it is respectively quicker and slower. The initial "over-reduction" is inhibited by the addition of NaCl and, as a rule, is not seen at all in analyses of serum filtrates after tungstate precipitation of the proteins.

The quantity of potassium ferricyanide reduced is, within wide limits, directly proportional to the quantity of uric acid if only a certain excess of potassium ferricyanide is assured. Further additions of potassium ferricyanide do not alter the ratio.

Brøchner-Mortensen has evolved methods for the quantitative analysis of uric acid in serum and urine (34) based on the investigations made of the reaction between uric acid and potassium ferricyanide.

In the serum analyses the proteins are precipitated with sulphuric acid and sodium tungstate. A definite excess of potassium ferricyanide was added to the filtrate and a buffer mixture, which ensures that the reaction takes place at pH 11. The quantity of potassium ferricyanide not reduced is determined by iodometric titration.

Definite quantities of uric acid added before or after the protein precipitation were recovered quantitatively.

The quantitative isolation of the uric acid in the serum filtrate was not possible by the usual methods, but in a series of experiments when serum filtrates were analysed, both with and without the addition of uric acid, an average 85 per cent of the amount of uric acid expected was recovered, just as large quantities of the uric acid added and of that found by direct analysis of the filtrate being lost by isolation. It is therefore probable that the reduction found by direct analysis is entirely due to uric acid. A series of experiments showed furthermore that the reduction found in the given analytical conditions cannot be due to glucose, creatine, creatinine, glutathion, ergothioncin, urea, glycine, cystine, xanthine, allantoin, guanine, sodium salicylate, phenol, ascorbic acid, or enzymes.

The mean error for the method is 2 per cent. The values found in comparative analyses average 1.5 mg per cent higher than those found by Folin's method (1933) (108).

A method for the analysis of uric acid in urine was elaborated on similar principles. Since urine, contrary to serum, contains, in addition to uric acid, measurable amounts of substances that reduce potassium ferricyanide in the given conditions, the uric acid was isolated by precipitation according to Salkowski's (363) principle. A certain constant loss of uric acid occurs during the isolation but, after correction for this, the quantity of potassium ferricyanide reduced is directly proportional to the amount of uric acid. A quantity of uric acid added was recovered quantitatively. The mean error for the method is about 2 per cent. The values found show fairly good agreement with those found by Folin and Shaffer's method (120) if the time of precipitation in the latter is prolonged to 48 hours (304).

URIC ACID IN THE BLOOD AND URINE OF NORMAL PERSONS

Uric acid in blood

As early as the middle of the last century it was known, especially through Garrod's studies (140) that the blood of patients with gout and uremia contains uric acid but, with the methods then available, uric acid could only be demonstrated in exceptional cases in the blood of normal subjects. Garrod (140) and a few subsequent authors (267, 323) certainly succeeded in just a few cases, though it was not until after the elaboration of Folin's colorimetric method that it became possible to demonstrate uric acid in the blood of all human

beings The quantity of uric acid found in the blood of normal subjects by the phosphotungstic acid method varied, however, very considerably with the different modifications of the method employed, the lowest values having generally been found with the older methods (Table 1)

Flatow (102), using the potassium ferricyanide method, found 4 ± 0.2 mg per cent with a mixed diet

Brøchner-Mortensen's method (34, 36) gave from 5.4 to 8.8 mg per cent for 33 normal men and from 4.4 to 8.1 mg per cent for 37 normal women on ordinary diet After at least 3 days on purine-free diet, the figures were from 3.3 to 8.4 mg per cent for 17 men and from 3.6 to 7.0 mg per cent for 15 women whose purine metabolism might be assumed to be normal (Table 2)

Fradà (126), using the same technic found from 4.1 to 5.9 mg per cent for 7 normal men and from 3.8 to 5.4 mg per cent for 7 normal women

No relation between the uric acid values found by the various methods and factors such as age, height, and weight has, as a rule, been observed Fairly high values have, however, been found in the newly-born (23, 218) Several authors have found higher values for men than for women (24, 34, 126, 202)

It was stated formerly that the uric acid in the blood was almost constant in the individual, independent of the composition of the diet (83, 88, 195, 238, 429) Many investigators, however, have found an increase in the content of uric acid in the blood, of temporary or longer duration, after the consumption of foods containing purines (4, 34, 60, 148, 219), and it is generally reckoned that the uric acid in the blood is about 1 mg per cent less with a purine-free than with an ordinary purine-containing diet (10, 34, 222, 296) Investigations by the potassium ferricyanide method in the cases of 8 men and 12 women whose uric acid metabolism might be presumed to be normal gave average values of 6.9 and 6.1 mg per cent, respectively, for ordinary purine-containing diet, and 6.1 and 5.3 mg per cent after purine-free diet for at least 3 days (34)

Very slight variation from day to day is observed with purine-free diet when conditions are otherwise uniform, generally not more than 1 mg per cent, and with no definite tendency to a decrease in the

TABLE 1

Uric acid in the blood of normal persons Comparison of the results obtained by various authors by the phosphotungstic acid and the potassium ferricyanide methods

Potassium ferricyanide method

YEAR	AUTHOR	METHOD	EXAMINED BY	URIC ACID	mg per cent		
				Ordinary diet	Purine-free diet		
				Number investigated	Range	Number investigated	Range
Phosphotungstic acid method				mg per cent		mg per cent	
1913	Folin and Denis (113)	Folin and Denis (112)	Blood	21	0.8 to 3.0		
1914	Steinitz (402)	Steinitz (402)	Blood	5	2.4 to 4.5	12	1.4 to 3.7
1917	Häst (195)	Häst (195)	Blood			17	<0.5 to 2.64
1923	Jeanbreaux, Christol and Nikellisch (205)	Grignaut (157)	Serum	25	3.0 to 5.6		
1924	Folin, Berglund and Derick (110)	Folin (105)	Plasma		4.6	6	3.0 to 6.0
1911	Jordan and Gaston (211)	Folin (107)	"Unlinked blood" Serum	13	2.4 to 5.8		
1911	Herafeld (186)	Herafeld (186)	Serum	?	8.0 to 12.0		
1914	Christiansen (139)	Folin (105)	Serum	43♂	3.2	34	2.8 to 5.8
1914	Berglund and Frisk (24)	Folin (108)	"Unlinked blood" Serum	89♀	2.7		
1919	Jacobson (202)	Folin (108)	Serum	63♂	4.4		
				37♀	4.0		
Potassium ferricyanide method				mg per cent		mg per cent	
1928	Flatow (102)	Flatow (101)	Serum	?	4.0		
1937	Brächner Mortensen (34)	Brächner Mortensen (34)	Serum	33♂	7.4	17♂	6.3
1918	Frølich (126)	Brächner Mortensen (34)	Serum	37♀	6.3	15♀	5.4
			Serum	7♂	4.7		
				7♀	4.4		

TABLE 2

Uric acid in the serum of normal subjects after at least 3 days' purine-free diet (potassium ferricyanide method)

NUMBER	AGE	HEIGHT	WEIGHT	URIC ACID IN SERUM
Men				
	<i>years</i>	<i>cm</i>	<i>kg</i>	<i>mg per cent</i>
1	31	168	51	4.0 (3.3 to 4.3)
2	50	171	58	4.5
3	60	165	53	5.0 (4.4 to 6.1)
4	51	161	62	5.2
5	28	174	60	5.4 (4.8 to 5.8)
6	33	176	66	5.9 (5.5 to 6.3)
7	14	155	40	6.0 (5.4 to 6.5)
8	48	155	66	6.1 (5.7 to 6.4)
9	20	174	62	6.1
10	38	178	77	6.5
11	57	165	66	6.9
12	22	164	70	6.9
13	16	158	50	7.3
14	19	178		7.3
15	26	167	57	7.6
16	41	166	74	8.0 (7.9 to 8.1)
17	29	183	84	8.1 (7.9 to 8.4)
Women				
1	41	158	53	3.9 (3.6 to 4.1)
2	65			4.4
3	28	161	51	4.7
4	18	174	58	5.0 (4.4 to 5.4)
5	37	157	55	5.1 (5.0 to 5.2)
6	36	152	48	5.2 (5.1 to 5.4)
7	32	168	61	5.3 (4.7 to 5.7)
8	38	155	62	5.3 (4.8 to 5.7)
9	33	162	80	5.4 (5.2 to 5.5)
10	31	157	53	5.5 (4.7 to 6.2)
11	33	154	54	5.8 (5.7 to 6.3)
12	36	163	57	5.8 (5.0 to 6.5)
13	50	165	67	5.9 (5.7 to 6.1)
14	39	168	74	6.1
15	15	158	39	6.6 (5.8 to 7.0)

values when the diet is of longer duration. Great variations, however, are found with ordinary mixed diet and, when a diet very rich in purines is given, a considerable increase in the content of uric acid

in the serum is seen to set in rapidly, for instance, a rise from 5.5 to 10.1 mg per cent in the course of 2 days (fasting morning values) (34)

The content of uric acid in the blood increases several mg per cent in the course of a few days during a longer fast, after which it remains constant for the whole of the period of the fast. Hoeffel and Moriarty (191) thus found by the phosphotungstic acid method a rise in from two to four days to an absolute value of from 8 to 12.2 mg per cent, Lennox (236) in five days a rise to 14 mg per cent.

A corresponding increase in the content of uric acid in the blood is observed with a high fat diet (173)

The ingestion of cinchophen and of salicylates decreases the content of uric acid in the serum (for further details see p. 186 et seq.)

Slightly increasing values are sometimes seen in serum when the excretion of uric acid decreases because the water output is lowered. This increase is not, however, constant and is generally moderate. If the lower renal excretion continues, no further rise is observed, presumably because of the increased extra renal excretion (34)

Repeated tests made during the same day generally show that the highest values are to be found in the morning. They are constant, or fall slightly, during the forenoon, show a distinct fall during the afternoon, and are lowest during the first part of the night, rising again during the second part. The variations are generally between 1 and 2 mg per cent. No changes are seen in the content of uric acid in the serum after purine free meals, though a rise of a couple of mg per cent occurs an hour or two after purine-rich meals (34, 136, 255)

The influence of muscular exercise on the content of uric acid in the blood has been investigated by various authors, nearly all of whom find greater or less increase during and after the activity. The extent of the exercise is generally stated most indefinitely, though it is almost always fairly severe.

Levine, Gordon, and Derick (240) examined marathon runners before and after the race, finding an increase of up to 2.4 mg per cent in the content of uric acid in the blood of the most exhausted participants. Rakestraw (336, 337) found that a short period of exercise (running up stairs) increased the content of uric acid in the plasma by up to 2.7 mg per cent (average of several observations 0.9 mg

per cent) Exercise that was less violent, but of longer duration (cycling for two or three hours) increased the content of uric acid in the plasma by up to 2.9 mg per cent (average 1.5 mg per cent) Repeated measurements made after work showed further increases (average 0.5 mg per cent for $1\frac{1}{2}$ hours) Lucke (263) found an increase of 1 mg per cent after work, while Quick (332) found no definite changes after walking or running

Uric acid excretion

The most important organs in the excretion of uric acid are the kidneys, through which a quantity that may vary enormously, but which generally amounts to from 200 to 800 mg, is removed every twenty-four hours A small amount is eliminated with the sweat (3, 361, 431) and a rather larger amount with the digestive secretions A somewhat varying amount is found in the saliva and in the gastric secretions, though it is generally about 1 mg per cent (230, 257, 278, 350, 404) A slightly larger quantity is found in the bile, most frequently though in a concentration that is a couple of mg per cent lower than that of the blood, the values in the cystic bile being somewhat higher (42, 43, 44, 175, 259, 274, 321) Information regarding the concentration of uric acid in the intestinal secretions is less certain (261, 383)

Lucke (263) reckoned from his own and from other studies that from 6 to 12 mg uric acid is generally excreted in saliva, from 15 to 20 mg with the gastric excretions, and from 20 to 30 mg with the biliary and pancreatic secretions, the total "enterotropic" quantity amounting to from 40 to 60 mg, i.e. only about 10 per cent of the "urotropic" excretion From another quarter, however, rather larger values are given (42, 43)

The "enterotropic" excretion is increased in hyperuricemia especially when this is due to a diminution of the renal functions, and, to a certain extent, it compensates the decrease in the "urotropic" excretion (230, 258, 260, 275, 276, 277)

Renal excretion of uric acid Opinions regarding the mechanism of the excretion of uric acid through the kidneys have varied somewhat in accordance with the opinions with reference to the renal function taken as a whole

It is not within the scope of the present study to give even a cursory summary of the ever changing views of the last fifty years regarding the renal functions. Mention will therefore merely be made in the following of a number of studies in which, particularly, the conditions of the excretion of uric acid have been investigated.

In agreement with the theory then generally prevalent, a series of studies appeared about the turn of the century, presuming to show that the uric acid is secreted through the renal tubules (7, 78, 92, 93, 287, 365).

The experiments performed generally consisted of the peroral administration of purine bases to, or the intravenous or intraperitoneal injection of uric acid into, test-animals such as dogs and rabbits that normally excrete very scanty quantities of uric acid. Subsequent investigation showed no changes in the glomeruli but fine-grained or needle-shaped precipitates in and among the cells of the convoluted renal tubules, especially in the part facing the lumen. No deposits were found in the cells of Henle's loop and the collecting tubules, but the so-called "spheroliths," increasing in size towards the collecting tubules, were found in the lumen. Analysis showed that these contained uric acid. The results of the experiments thus agreed splendidly with those of the many previous and contemporary investigations into the excretion of dyes regarded as evidence of the secretion of these substances.

Subsequent authors (81, 288, 406), however, asserted that the facts found by histological investigation might just as well indicate that the uric acid is eliminated in the glomeruli and is then partly re-absorbed or re-diffused in the tubules. This is indicated, according to Cushny (81), by the fact that uric acid crystals are also found between the cells. "Spherolith" formation was said to be due to the precipitation of uric acid caused by the absorption of water. Later on, uric acid was also successfully demonstrated in the glomerular capsule by means of a slightly different technic (143, 384, 385).

Since the problem can hardly be solved by purely histological investigation, a long series of more physiological studies has been carried out during recent years.

A number of perfusion experiments were performed with frogs' kidneys, the double vascular supply of which it was considered would

provide a possibility of differentiating the functions of the glomeruli and the tubules. While the blood from the renal artery passes through the glomerular loops and then the peritubular capillaries, the blood from the renal portal vein of the frog goes direct to, and only supplies, the peritubular capillaries.

According to Richards and Walker (343), however, a reflux of blood from the tubular capillaries to the glomeruli may occur fairly often. In order to check this source of error in the experiments Lueken (264) added a colored substance, cyanol, to the fluid that is conducted through the renal portal vein. Since cyanol, it is alleged, can only be excreted through the glomeruli and not through the tubules, it may be supposed that in cases in which the urine is colorless the reflux to the glomeruli asserted by Richards and Walker (343) has not taken place.

In some of his experiments Lueken (264) perfused the artery with Ringer's solution, to which 2.6 mg per cent uric acid had been added, and the vein with pure Ringer's solution. The urine excreted after this contained from 6 to 6.2 mg per cent uric acid. The concentration index (the ratio of the content of uric acid in the urine and that in the "blood") was from 2 to 2.5, the clearance (the concentration index multiplied by the urine volume in cc per hour) about 4 cc per hour. If the cellular function were now paralyzed by the addition of cyanide to the venous perfusion the concentration index was lowered to from 1 to 1.5, and the clearance to about 2.5.

On the other hand, when the artery was perfused with pure Ringer's solution and the vein with a uric acid solution, the concentration index was found to be about 10 and the clearance from 2 to 4.5. When cyanide was added to the venous perfusion the concentration index fell to from 1.5 to 5 and the clearance to about 1. The addition of cyanide as a rule caused a minimal increase in the water output. After the paralysis of the tubules had ceased the function was completely or partially restored.

Lueken concluded from these experiments that uric acid is excreted through the glomeruli and that the tubular cells concentrate the uric acid present in the lumen from 2 to 2.5 times. This tubular function partially disappears on the addition of cyanide. In the other group of experiments, in which the uric acid is supposed to reach the tubules from the vascular side only, it is, on the contrary, concentrated about

tenfold, from which the author concluded that the concentration of uric acid in the tubules is due, to a greater extent, to the secretion of uric acid and, to a lesser extent, to the reabsorption of the liquid

The difference between the two sets of concentration indices is, however, largely due to the fact that the urine volume was considerably lower in the second than in the first. The figures for clearance also show somewhat lower values for the second group than for the first. The whole of the evidence stands or falls, furthermore, on the correctness of the assumption that the addition of cyanol can check the above-mentioned refluxion of the venous perfusion to the glomerules

A number of perfusion experiments were made with heart-lung-kidney preparations employed, in particular, by Starling and Verney (401). In these a constant circulation of the blood could be maintained and the pressure, the speed of circulation, and the composition of the perfusion liquid could be varied freely. The urine obtained in these experiments to a certain extent resembled normal urine, in which a number of various substances were concentrated in varying degrees. Cyanide poisoning caused the composition of the urine to resemble an ultrafiltrate of plasma.

Gremels and Bodo (154) carried out two sets of experiments. In the first, uric acid was injected into heart lung-kidney preparations from dogs. The water output was hereby increased a little, and the uric acid was excreted, the amount that had disappeared from the blood circulation being recovered approximately quantitatively in the urine. The clearance was from 3 to 6, independent of the urine output (over 0.7 cc./min). The secretion of urine ceased entirely when the blood pressure was lowered from 115 to 35 mm Hg. At this low blood pressure a quantity of uric acid injected remained unchanged in the circulating blood.

In the second series of experiments $N/200$ cyanide was added to the perfusion fluid. The water output remained almost unchanged, the concentration index, which was from 5 to 10, fell to about 1, and the clearance, originally from 5 to 10, to about 2.

The authors considered that they could conclude from these experiments that a small amount of uric acid was ultra filtered in the glomeruli and a large quantity secreted in the tubules.

The fact that the uric acid in the circulating blood remained un-

changed when the blood pressure was lowered, however, definitely contradicts the secretion theory, and the decreased excretion of uric acid after cyanide poisoning is a natural consequence of the diminished glomerular filtration caused by the increased pressure in the canals, which must occur when the absorption of liquid in the tubules is lowered

Such large quantities of glomerular filtrate have been collected of recent years by puncture of the functioning glomeruli of frogs and snakes that it has been possible to make quantitative analyses. The method was originally published by Wearn and Richards (435) and has since been improved by many investigators, a considerable number of sources of error having been removed.

Bordley and Richards (30) showed in 1933 that the content of uric acid is uniform in the plasma and glomerular filtrate of frogs and snakes after the administration of uric acid. From their experiments they thought they could calculate that the amount of the glomerular filtrate was of such size that the total amount of uric acid appearing in the urine might be due to ultrafiltration in the glomeruli, a theory that depends in the first place on the correctness of the assumed total number of active glomeruli.

Marshall (272), on the other hand, calculated the amount of the filtrate by glucose determination of the plasma and the urine (collected by urethral catheters) of lizards after the administration of phlorizin, and found that it was so small that less than 10 per cent of the uric acid in the urine could be due to ultrafiltration in the glomeruli.

Thus the facts are not quite obvious. The whole problem cannot, of course, be solved solely on the basis of uric acid investigations, but only in conjunction with other studies of the physiology of the kidneys. It must, furthermore, be emphasized that human beings have a special position in the animal kingdom as regards uric acid metabolism and that the human mechanism for the excretion of uric acid may, therefore, also possibly deviate from that found in the test-animals employed.

However, from all the evidence available it is presumably certain that an ultrafiltration of uric acid occurs in the glomeruli. But it is an open question whether the quantity of liquid filtered in the glomer-

uli is so large that it can contain the total amount of uric acid appearing in the urine, even though it be assumed that a certain reabsorption takes place in the tubules—or whether the quantity of the filtrate is so small that it must be assumed that secretion takes place in the tubules

From the most recent studies, however, it seems probable that the amount of glomerular filtrate in normal human beings is in the neighborhood of 100 cc per minute. If this proves to be true, and if all uric acid in plasma is freely filtrable, it may be assumed that between 90 and 95 per cent of the quantity of uric acid filtered is reabsorbed in the tubules (36, 37, 138)

The question of the ultrafiltrability of uric acid has been the subject of many investigations. It has been asserted that some of the uric acid in the blood appears in a colloid or combined, non-ultrafiltrable, form (24, 31, 85, 367). This is, however, hardly the case, since uric acid has been found by dialytic experiments with Rona-Michaeli's osmotic compensation method (59, 60) to be freely diffusible, furthermore, the same concentration of uric acid in plasma and in ultrafiltrate has been found by ultrafiltration experiments (2, 279, 396). Finally, as has been mentioned, the same concentration of uric acid in plasma and in puncture liquid has been found by glomerular puncture (30).

Dependence of the excretion of uric acid on the content of uric acid in the blood. Augmented excretion of uric acid is generally found in normal persons with an increasing content of uric acid in the blood (4, 6, 24, 34, 60, 110, 433). The examination of a number of normal persons by Brøchner Mortensen (34) showed that the coefficient of correlation between the content of uric acid in the serum and the quantity of uric acid excreted per minute was $+0.51 \pm 0.09$, while Gårdstam (139), on the contrary, found no definite relation between the two amounts.

Since the spontaneous variations in the uric acid content in the blood of individuals are very small, Brøchner-Mortensen (34, 37) carried out a series of determinations of the content of uric acid in the serum of 10 test persons and of the amount of uric acid excreted during short periods of from 30 to 60 minutes before and after the ingestion of purine rich meals, or the intravenous injection of lithium

urate The results of the investigation are shown in Figure I, in which the ordinate shows the amount of uric acid excreted per minute and the abscissa the concentration of uric acid in the blood. The values thus plotted are grouped about a straight line which does not pass through the zero point but cuts the abscissa at a point corre-

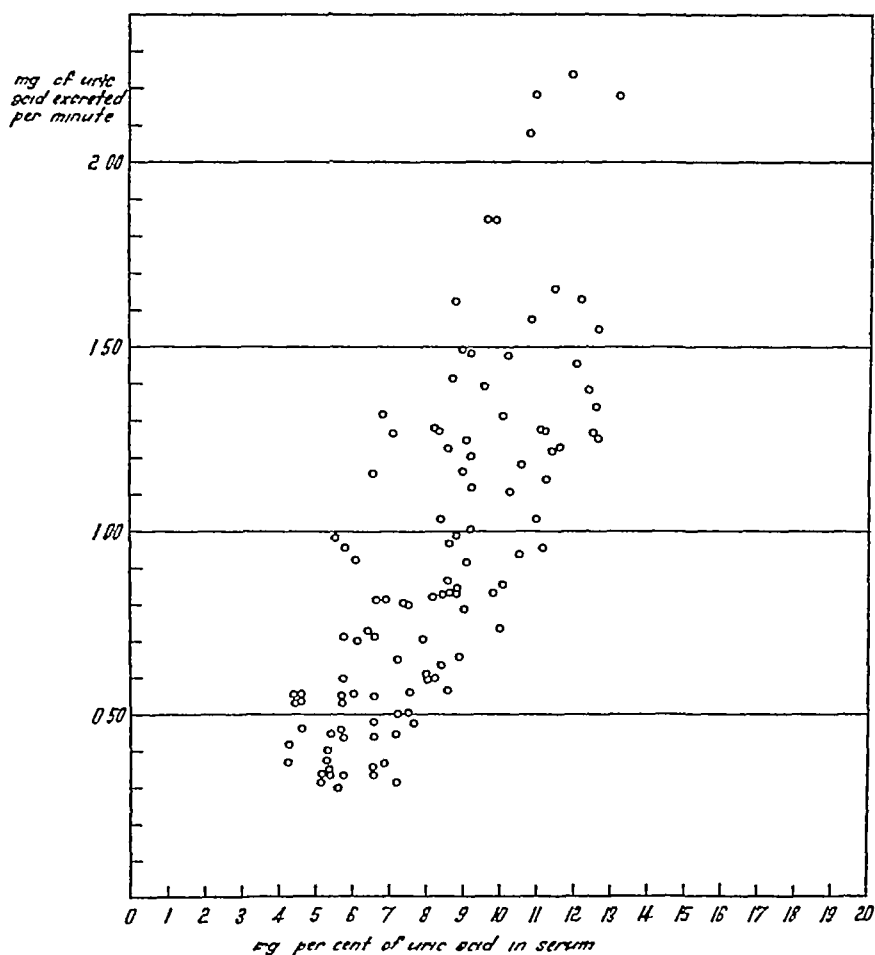


FIG I RATIO BETWEEN THE URIC ACID CONTENT OF SERUM AND THE QUANTITY OF URIC ACID EXCRETED PER MINUTE

sponding to about 4 mg per cent, contrary to what is found in the case of, for instance, urea

This may be thought to be due to various causes if the conception of the renal function is based on the filtration-reabsorption theory

It is, of course, feasible that the values for the content of uric acid

in the serum found by the ferricyanide method include substances other than uric acid. But this is hardly probable (34), and there can, at any rate, hardly be any question of an analytical error so large that it can explain the facts found. For, if the amount that lies between the zero point and the point of intersection represents substances other than uric acid, only a very small fraction of the uric acid found in many of the tests before administration would be "genuine." Furthermore, the variations found in the uric acid concentration of serum and in the amount of uric acid excreted do not always coincide. In many cases the excretion of uric acid increases very rapidly, while the increase in the blood occurs more slowly at first. Vice versa, decreasing excretion is observed later on in the tests, in spite of a continued increase in the blood.

For the same reason the explanation of the findings cannot be that only a certain part of the amount appearing before the administration is ultrafiltrable while the quantity added is freely filtrable. A large number of investigations also indicate, as has been mentioned, that the uric acid in the serum is freely filtrable (2, 30, 59, 60, 279, 396).

It is, however, probable that there is a renal threshold for uric acid as there is for glucose, chloride, phosphate, bicarbonate, ascorbic acid, creatine, amino acids, etc., varying with the individual but generally corresponding to a value of from 4 to 5 mg per cent uric acid in serum. Since, moreover, a lower concentration of uric acid may be found in urine than in serum, by both the phosphotungstic acid (139, 254, 415) and the potassium ferricyanide methods (34, 37), it must be assumed that the uric acid is reabsorbed actively in the tubules. About 95 per cent of the amount of uric acid filtered in the glomeruli seems to be reabsorbed in normal persons on a purine free diet, or fasting, and from 80 to 90 per cent after the ingestion of purine (36, 37).

The changes in the excretion of uric acid found after this ingestion can, however, hardly be due to the presence of a fixed threshold alone, since the changes in the concentration of uric acid in serum and the quantity of uric acid excreted in the time unit, as has been mentioned, do not coincide.

A more detailed explanation of the facts found can hardly be given with our present knowledge and conception of the function of the tubules.

Since uric acid has usually been considered to be a waste product, it has, from the teleological point of view, been rather difficult to accept the theory that uric acid is reabsorbed actively in the tubules. Rehberg (339) therefore propounded the hypothesis that the urate ion followed the sodium ion passively when the latter was actively reabsorbed in the tubules.

This hypothesis has, however, not yet been supported by experiments, though, as mentioned below, increased excretion of uric acid has been found after the ingestion of sodium bicarbonate and pyruvic acid, and decreased excretion during fasting, high fat diet, and the ingestion of CaCl_2 , lactic acid, and aromatic acids. By feeding gouty patients with high fat diets, some experiments seem to indicate that the reabsorption of uric acid in the tubules rises up to 99 per cent of the amount filtered (36).

Dependence of the excretion of uric acid on the water output The relation between the water output and the amount of uric acid excreted is a problem that has engaged the attention of many authors. Kennaway (215) found that the excretion decreased as the urine volume increased, while Robertson (352) and Morris and Rees (307) found from their experiments with chickens, rats, and rabbits (after the injection of uric acid) increasing excretion with increasing water output. The same was found in man by Chabanier and Lobo-Onell (60).

Most investigators, however, think that the quantity of uric acid excreted is independent of the excretion of urine (5, 24, 139, 195, 434). Berglund and Frisk (24) thus state that the coefficient of correlation for the amount of urine and that of uric acid excreted in one hour is $+0.21 \pm 0.11$. No figures, however, are given for the individual urine volume. Brøchner-Mortensen (34) examined a large number of test-persons whose excretion of uric acid might be considered to be normal and found from experiments made at periods of 24 hours and of from 30 to 60 minutes that the quantity of uric acid excreted during the unit of time was constant, and was independent of variations in the urine volume if the latter was above a certain amount—the augmentation limit—, about, or a little less than, 1 cc per minute. The amount of uric acid excreted when the water output is lower is approximately proportionate to the square-root of the urine volume excreted during the time-unit (Fig. II).

The same author calculated the augmentation limit for 6 normal persons in accordance with the directions laid down by Eggert Møller, McIntosh and Van Slyke (289) and found values corresponding to a urine volume of 1.08, 0.96, 0.71, 0.64, 0.54, and 0.52 cc. per minute respectively, i.e. considerably lower than the augmentation limit for urea (34)

Corresponding figures may be obtained by re-calculating the results of many previous investigators (139, 248, 249, 286, 315, 393, 394)

Recognition of this fact is, as will be seen below, of decisive importance in estimating the value of the results from studies of the variations in uric acid excretion in various conditions

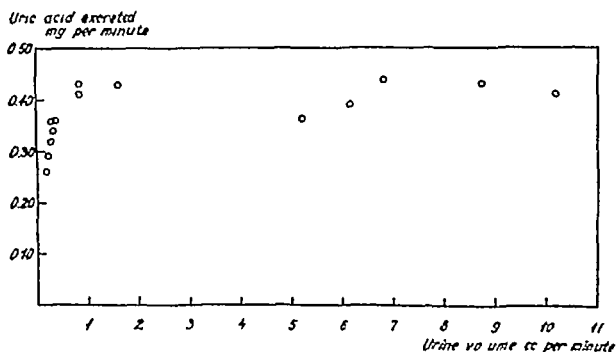


FIG. II. DEPENDENCE OF THE EXCRETION OF URIC ACID ON THE WATER OUTPUT

Furthermore, sudden great changes in the excretion of urine seem to be able to some extent to exert a particular influence on the amount of uric acid excreted. If the excretion of urine is suddenly increased, the amount of uric acid excreted during the unit of time will be found to be considerably above the average found for the individual. Vice versa, if the excretion of urine suddenly decreases considerably, lower values will be found for the uric acid excreted than would be expected, even though the augmentation limit be taken into consideration (34)

The most obvious explanation of this would be that the urine excreted during the period is mixed with a certain amount of urine from the previous period, but against this must be put the fact that a corresponding simultaneous change in the excretion of creatinine does

not necessarily appear (34) Considered from the point of view of the filtration reabsorption theory this must be presumed to be due to a change in the reabsorption of uric acid in the tubules Corresponding conditions have been observed in some investigations of urea excretion (32)

Variations in the amount of uric acid excreted Studies of the human excretion of uric acid in normal and in pathological conditions gave extraordinarily varying results until, about the beginning of the century and particularly as a result of a series of studies made by Burian and Schur (50, 51, 52, 53, 54, 55) and Sivéu (389, 390), the separation into two parts of the amount appearing in the urine was reached These were, the *exogenous*, originating in the purines ingested with the food and varying proportionately with their amount, and the *endogenous*, which is excreted by a person on a purine-free diet

The quantity of endogenous uric acid is generally from 250 to 600 mg per day It is fairly constant in the individual, even with a purine-free diet of long duration (283), and was originally considered to be quite independent of the composition of the purine-free diet

Subsequent studies have, however, shown that variations in the composition of the diet considerably affect the amount of endogenous uric acid excreted

Folin (104) thus found a decrease of up to 50 per cent in the uric acid excretion of a number of test-persons when a change was made from a purine-free diet containing about 19 g to one containing about 1 g nitrogen The results of these experiments were confirmed by Leathes (233), who observed a fall of 30 per cent in similar circumstances, by Taylor and Rose (410), who found an excretion of 0.3 g uric acid a day for ordinary purine-free diet and a considerable rise (up to 0.82 g) after 40 g nitrogen (white of egg) had been added to the diet, and by a great many subsequent authors (110, 195, 237, 238, 242, 296, 335, 357)

The results of many of these experiments have, however, been misinterpreted, no notice having been taken of the above-mentioned dependence of the uric acid excretion on the water output More exact examination of those studies in which the data given are sufficient to permit a calculation to be made often shows that the varia-

tions in the quantity of uric acid excreted were entirely or partially due to simultaneous changes in the water output, the urine volume, and thereby the excretion of uric acid, having been lowest when the food contained least protein, and vice versa.

In some of the experiments the urine volume was, however, so relatively constant from period to period that it is permissible to conclude from the investigations that the amount of uric acid excreted is dependent on the composition of the purine-free diet (104, 195, 237, 238)

Leopold, Bernhard and Jacobi's experiments (238), however, show that the addition of protein to the diet only causes a temporary increase in the excretion of uric acid. It must, furthermore, be pointed out that the "protein-poor" diet in Folin's experiments (104) only contained about 1 g protein-N a day, so that there was no nitrogen-equilibrium. In all probability the variations found are not specific for the excretion of uric acid, for corresponding changes in the excretion of urea and of creatinine have been found when the content of protein in the diet is varied (75, 95, 147)

The amount of uric acid excreted varies somewhat during the 24 hours of the day. Sivéén (389) found that the excretion was greatest in the forenoon and least at night, and Leathes (233) found excretions of 360 and 260 mg by day, and of 250 and 160 by night, respectively, for two different purine-free diets

If no food is ingested, the excretion of uric acid remains fairly constant during the forenoon, after which it falls almost uniformly during the course of the day. It is lowest at night, rising again to its initial values the next morning (34, 243, 286, 315, 393, 394). Somewhat the same course is observed when the diet is purine free, though some rise in the excretion may be noticed in connection with meals (34, 195, 241, 255, 285)

This is largely due to variations in the excretion of urine but, if the values for the experimental periods in which the excretion of urine is over 1 cc/min (the uric acid augmentation limit) only are included in the calculation, it will be found that in many of the investigations about 0.4 mg uric acid is excreted per minute during the morning hours, after which the quantity decreases to about a half during the day (34, 241, 243, 293, 294, 315)

The influence of individual foods on the excretion of uric acid, measured for shorter periods, has been the subject of numerous investigations

However, the changes found in many of these experiments were caused, entirely or partially, by simultaneous variations in the urine output

A definite increase in the excretion of uric acid is generally seen after the ingestion of foodstuffs that are rich in purines, and this increase occurs even within the first hour after a meal (34, 269, 285, 293, 294)

Some of the studies show that there are no definite changes after the ingestion of purine-free proteins (34, 285), others show a definite increase in the excretion of up to 100 per cent (241, 243, 293, 294) No changes in the excretion of uric acid that cannot be attributed to simultaneous changes in the excretion of urine are seen in any case after the ingestion of fat (34, 241) The same is generally the case after the ingestion of carbohydrates (34, 241, 286), but a fairly distinct increase in the excretion of uric acid, which, however, never even approximates the changes observed after the ingestion of protein, is seen in a few experiments (294) after the ingestion of very large quantities, e g several hundreds of grams of honey

A considerable decrease in the excretion of uric acid occurs immediately during a fast of long duration, the value rises again to almost the usual endogenous level after a few days Benedict (13) thus found an initial fall to 0.042 g uric acid N followed by a fairly constant excretion of 0.1 g a day Lennox (236) found an initial fall to 0.1 g uric acid followed by a constant excretion of 0.3 g a day Corresponding values have also been found by many other investigators (40, 237, 253, 381)

A similar decrease in the excretion of uric acid is observed with high fat diet (56, 172, 173, 422), after the administration of CaCl_2 (1, 400), lactic acid and aromatic acids (331), while, on the other hand, an increased excretion has been described after the administration of bicarbonate (298, 382, 387, 422) and pyruvic acid (331)

Cinchophen generally produces a considerable increase in the excretion of uric acid and a simultaneous decrease in the uric acid in the blood of normal subjects and gouty patients (24, 87, 90, 97, 116, 128, 138, 150, 151, 152, 156, 182, 249, 281, 297, 317, 395, 399, 400, 402, 437, 438, 442) No, or only a slight, increase in excretion is seen in a

few cases (116, 152, 156, 297, 395), but, as a rule, the excretion of uric acid increases greatly soon after the ingestion, slightly after continued ingestion, and becomes subnormal after the ingestion ceases (97, 152, 400)

The cause of the increased excretion is, presumably, a decrease in the reabsorption in the tubules, since the excretion of other non-protein nitrogen substances and of water is, as a rule, unchanged. Simultaneously, an increased flow of uric acid seems to occur from the tissue to the blood. A primary increase in the blood uric acid is described in a few cases (156, 249). Denervation of the kidneys of Dalmatian dogs stops the action of cinchophen (150, 151).

Salicylates produce a moderate increase in the excretion of uric acid of normal subjects and patients with gout and a slight corresponding decrease in the uric acid in the blood (86, 98, 169, 207, 353, 382). The action is increased considerably by the simultaneous ingestion of glycine (330).

Sodium benzoate was found by *Demis* (87) to increase the excretion of uric acid, though other authors found the excretion to be unchanged or lessened (244, 329, 407).

Piperazine produces a slight increase in the excretion (49, 90).

Colchicine produces no increase of the urotropic excretion (36, 87, 273), but some increase in the enterotropic excretion has been found in experiments with animals (383).

Caffeine and *theophyllin* have been found by several authors to increase the excretion of uric acid (24, 171, 314, 358). This is largely due, however, to the fact that these substances themselves react with many of the uric acid reagents employed in the colorimetric methods (171, 314). *Theobromine* causes no increase in the excretion of uric acid, as it does not react with uric acid reagents.

The *Carlsbad cure* produces increased excretion of uric acid (405), though it is hardly so great that it cannot be attributed to the increased excretion of urine.

Adrenalin produces very varying conditions. Some authors find increased excretion of uric acid (62, 168, 177). Others find decreased excretion (228) or indefinite changes (248). Similar indefinite or contradictory results are found after *pilocarpin* and *ergotamine* (168, 177, 269, 286).

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Sodium benzoate was found by *Denis* (87) to increase the excretion of uric acid, though other authors found the excretion to be unchanged or lessened (244, 329, 407).

Piperazine produces a slight increase in the excretion (49, 90).

Colchicine produces no increase of the urotropic excretion (36, 87, 273), but some increase in the enterotropic excretion has been found in experiments with animals (383).

Caffeine and *theophyllin* have been found by several authors to increase the excretion of uric acid (24, 171, 314, 358). This is largely due, however, to the fact that these substances themselves react with many of the uric acid reagents employed in the colorimetric methods (171, 314). *Theobromine* causes no increase in the excretion of uric acid, as it does not react with uric acid reagents.

The *Carlsbad cure* produces increased excretion of uric acid (405), though it is hardly so great that it cannot be attributed to the increased excretion of urine.

Adrenalin produces very varying conditions. Some authors find increased excretion of uric acid (62, 168, 177). Others find decreased excretion (228) or indefinite changes (248). Similar indefinite or contradictory results are found after *pilocarpin* and *ergotamin* (168, 177, 269, 286).

The influence of *exercise* on the excretion of uric acid has been the

subject of various investigations, the results of which, on the whole, agree fairly well. Burian (51) found that the excretion of uric acid falls somewhat during work and rises after work, the variations being to some extent compensatory. Similar observations have been published by many subsequent authors (153, 180, 206, 215), though others find that the total excretion is somewhat increased (104, 141, 263). Quick (332) found that the excretion of uric acid during rest in the forenoon remains constant at about 25 mg per hour. Walking at an ordinary pace does not change this, but strenuous work or running sometimes, but not always, decreases the excretion to about a half.

The details given in most of these studies are not full enough for an estimate to be made of the extent to which the variations observed in the excretion of uric acid are due to variations in the water output. A few more fully described experiments (153) show that the decrease in the excretion of uric acid during exercise coincides with a fall in the urine volume to below the augmentation limit. The increased excretion after exercise is probably connected with the frequently observed increase in the uric acid in the blood (see above). Variations in the lactic acid metabolism in the organism perhaps play a certain part in the changes (332).

Uric acid clearance Many authors have tried to form a basis for their estimation of the abilities of different persons to excrete uric acid by comparing the content of uric acid in the blood and that in the urine.

Watanabe (434) thus found that the ratio between the content of uric acid in the blood (in mg per cent) and the amount of uric acid excreted in 1 hour (in mg) was constantly from 0.10 to 0.30 for normal persons, but that it is higher for patients with renal insufficiency. Steinitz (403) calculated the ratio of the content of uric acid in the blood (in g per liters) and the square root of the quantity of uric acid (g) excreted in 24 hours and found values between 0.037 and 0.051 for normal persons, but that the figures were considerably higher for patients with Bright's disease and gout. Similar investigations with corresponding results were carried out by Highley and Upham (188) and by Chabanier and Lobo-Onell (60). Gottlieb (149) calculated the ratio between the uric acid concentration in urine and that in blood after dry diet for 24 hours and found values about 20 for nor-

mal persons and considerably lower figures for patients with renal insufficiency

Berglund and Frisk (24) worked out the so-called "Elimination Index," the ratio between the amount of uric acid excreted in 1 hour (mg) and the content of uric acid in the blood (mg per cent). The elimination indices for 76 normal persons were found to vary from 5.1 to 19.2 (average 11.4). Lower values were found for patients with renal insufficiency.

Finally, Brøchner-Mortensen determined the uric acid clearance, using the formulae found from the investigation of urea. In view of the above-mentioned influence of the water output on the excretion of uric acid, the formula for maximum clearance was used for major urine volumes (> 1 cc per minute) and that for standard clearance for lower urine volumes (< 1 cc. per minute).

The average maximum uric acid clearance in 84 determinations involving 16 normal persons and made in standard conditions varied from 5.10 to 9.00, i.e. between 74 and 130 per cent of the common mean figure 6.93. The individual values varied from 3.5 to 10.2, i.e. between 51 and 147 per cent of the common mean figure. The average figures for uric acid standard clearance in 42 determinations involving 8 normal persons varied from 3.95 to 8.78, i.e. between 56 and 124 per cent of the common mean figure 7.08. The individual values varied from 3.7 to 11.7, i.e. between 52 and 165 per cent of the common mean figure. Comparative tests showed that the uric acid clearance varied from 3.7 to 7.8 (average 6.4) per cent of the creatinine clearance (urine volume > 1 cc per minute) and from 6.7 to 13.4 (average 10.1) per cent of the urea clearance (urine volume > 2 cc. per minute). Calculations of the uric acid clearance of Gårdstam's (139) and of Berglund and Frisk's (24) material show 11.9 and 19.1 as mean figures, respectively. The difference between the values in the various studies is due to the various analytical methods employed.

There does not seem to be any relation between the size of the uric acid clearance and factors such as sex, age, height, and weight, and there does not seem to be any measurable difference in the values if the test persons are given a purine free diet or an ordinary purine containing diet in the days preceding the investigation.

Several consecutive determinations of the uric acid clearance dur-

ing the forenoon show that the variations found are in no way related to the period at which the determinations were made Tests made at

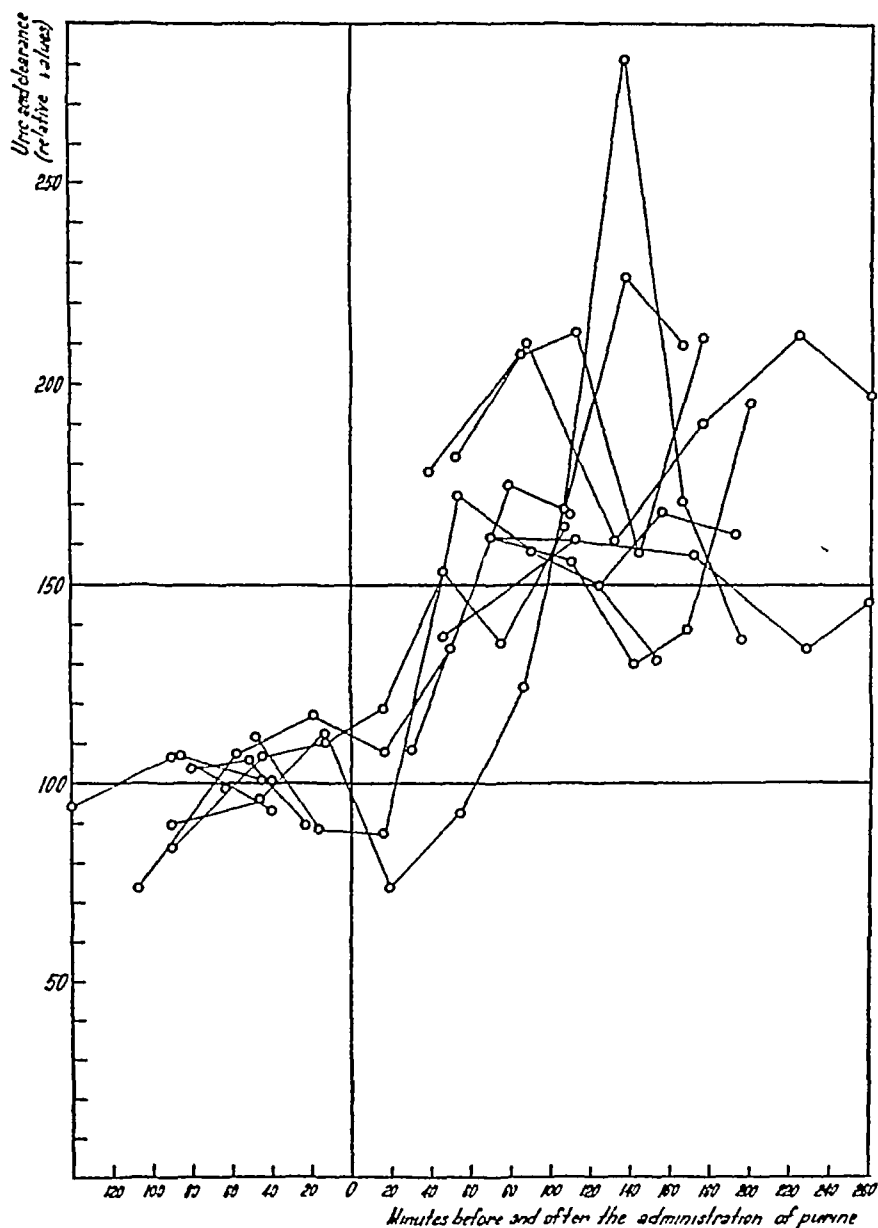


FIG. III URIC ACID CLEARANCE BEFORE AND AFTER ADMINISTRATION OF PURINE IN PERSONS WHOSE URIC ACID METABOLISM MUST BE CONSIDERED NORMAL

The values are recorded as percentage of the individual mean value determined before the administration

short intervals throughout the 24 hours showed mainly falling values for the uric acid clearance during the course of the day, the values being lowest at night and rising in the early morning. No variation in the uric acid clearance was observed as a rule after the ingestion of purine free meals containing proteins. A considerable increase was seen after purine-containing meals, especially when the meal was given in the forenoon. The same was also seen after the intravenous injection of uric acid (34)

When normal persons who for some days before the test have been on purine free diet or an ordinary purine-containing diet are given foods rich in purines or receive intravenous injections of lithium urate, the uric acid clearance is seen to increase by at least 60 per cent, often more (up to 180 per cent) (Fig III). The rise appears in one or two hours in the case of peroral, and more quickly in the case of intravenous, administration, generally lasting for several hours. A rise of from 50 to 85 per cent appears a little later and lasts a little shorter if the persons have been on a diet very rich in purines for the day before the test (36, 38)

The variations in the uric acid clearance found after administration seem to be due to changes in the reabsorption in the tubules. From the previous discussion it is, however, hardly probable that the variations are solely due to the existence of a definite threshold for uric acid.

If a 'corrected clearance' is calculated by subtracting the 'threshold value' found for each individual from the concentration of uric acid in the serum, the individual values before and after administration are only approximately constant, being generally about 30 cc., and varying from 10 or 15 to 80 or 90 cc (37)

URIC ACID IN THE BLOOD AND URINE IN GOUT

Uric acid in blood

As early as the middle of the nineteenth century Garrod (140) demonstrated the presence of uric acid in the blood of gouty patients by means of the thread test, though none could be demonstrated in the blood of normal subjects. After the elaboration of Folin's colorimetric methods, in 1912, uric acid was found in the blood of all human beings. In spite of the very varying results obtained by the different

modifications of the method, however, all the authors agree that increased values are found in the majority of gouty patients

Folin and Denis (115) were even of the opinion that hyperuricemia with no simultaneous increase of urea in the blood was a sure criterion for the diagnosis of gout

In 1917 McClure and Pratt (280) summarized the determinations made with Folin and Denis's method that had been published and found less than 3 mg per cent uric acid in the blood of the majority of normal persons and more than 3 mg per cent in that of 86 per cent of gouty patients

Of more recent authors, Chauffard, Brodin, and Grigaut (65) found from 4 to 5 mg per cent in normal persons and from 7.2 to 12.7 mg per cent in 13 gouty patients. Chauffard (64) carried out supplementary investigations and found corresponding values in a further 14 patients, the average value for all 27 patients thus being 9.4 mg per cent. Folin, Berglund, and Derick (110) found from 3.0 to 6.0 mg per cent for normal subjects and from 5.1 to 10.7 mg per cent for gouty patients on purine-free diet. Françon (127) found from 4 to 5 mg per cent in the blood of normal persons and from 6.8 to 11.5 mg per cent in that of gouty patients. Jordan and Gaston (211) found less than 4 mg per cent as a rule, though sometimes up to 5.8 mg per cent, in normal subjects, and from 3.9 to 10.2 mg per cent in 17 gouty patients. Schmidt (379) investigated a large number of patients with no changes in their uric acid metabolism and found an average 3.53 mg per cent uric acid in the blood, the average for 71 gouty patients was 5.83 mg per cent. Jacobson (202) investigating normal persons on mixed diet found from 1.9 to 6.7 mg per cent uric acid in the serum, the investigation of 17 gouty patients (untreated) showed from 6.0 to 13.7 mg per cent for purine-free diet and from 6.6 to 14.8 mg per cent for purine-containing diet.

Brøchner-Mortensen (34, 36, 38), using the potassium ferricyanide method, found from 3.3 to 8.4 mg per cent uric acid in the serum of normal persons on purine-free diet and from 6.0 to 22.1 mg per cent in gouty patients (Fig IV)

Although accumulations of uric acid are thus generally found in the blood of gouty patients, normal or even low values are very often reported (36, 38, 64, 65, 72, 110, 131, 161, 179, 184, 211, 247, 366)

Among the more recent authors Gudzent (162) found hyperuricemia in 18 out of 26 patients, Hench, Vanzant, and Nombard (184) in 72 out of 100, Cohen (72) in 39 out of 48, and Hill (189) in 84 out of 91 Bröchner-Mortensen, using the potassium ferricyanide method, found hyperuricemia in 43 out of 54 patients

Normal values for the uric acid in blood are not infrequently found, particularly in the initial stages of the disease, though hyperuricemia is observed almost constantly when the disease is a few years old (Fig V)

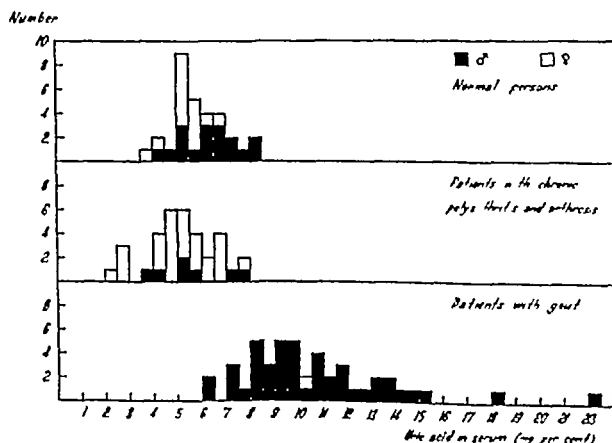


FIG IV URIC ACID CONTENT OF SERUM IN NORMAL PERSONS, PATIENTS WITH CHRONIC POLYARTHRITIS AND ARTHROSIS, AND GOUT

No definite variations in the content of uric acid in serum are found, as a rule, corresponding to the attacks, but in some cases, particularly in the initial stages, increased values are found during the attacks, and lower values during the intervals (36, 38, 72, 162, 183, 185, 328). No relation is found on the whole between the degree of the hyperuricemia and the frequency and severity of the attacks (36, 38, 247).

Hyperuricemia generally occurs in patients with tophi but there is no fixed rule (36, 38, 131, 179, 292).

Some decrease in the values is occasionally seen after purine free diet for a long time (77, 265).

Uric acid in urine

It has generally been considered that the hyperuricemia of gouty patients is of renal origin. This assumption was originally put forward by Garrod (140), who found that less uric acid was excreted by gouty patients than by normal persons, and that post-mortem examination often showed pronounced changes in the kidneys. Subsequent investigations have, however, shown that these renal changes are not constant and, at the beginning of the century, attempts were

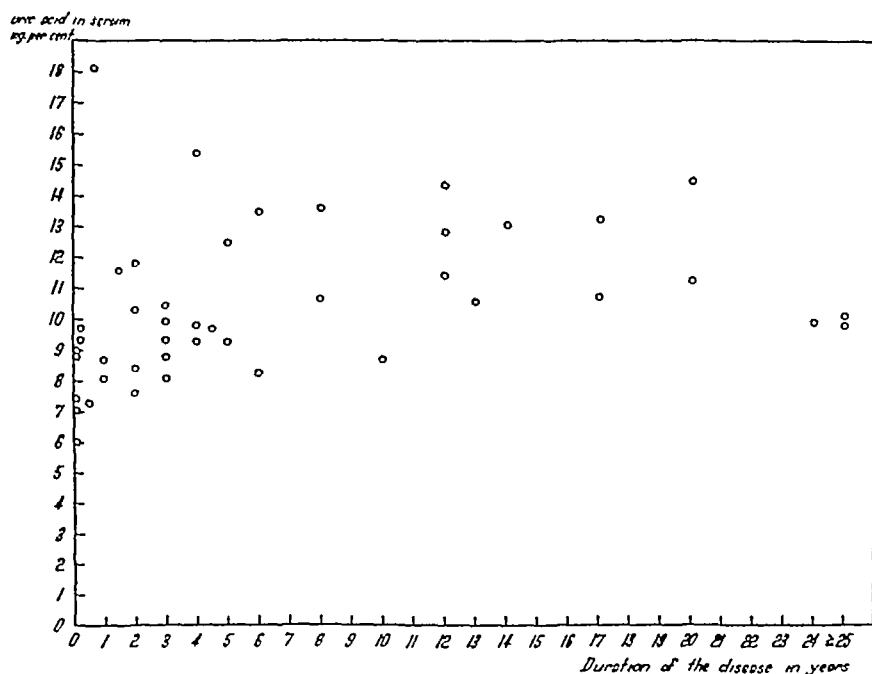


FIG. V. RELATION BETWEEN THE DURATION OF GOUT AND THE CONTENT OF URIC ACID IN SERUM (PURINE-FREE DIET)

made to explain the pathogenesis of the disease by theories of disturbance in the intermediary purine metabolism, or disturbances in the passage of uric acid from the blood to the tissue, and vice versa. The renal theory was, however, taken up again in a somewhat different form by Thannhauser (415, 417), who divided his cases into two groups, those with secondary renal gout, originating in an organic disease of the kidneys, and those with primary constitutional gout, in which the accumulation of uric acid in the organism may be presumed to be due to a partial functional decrease in the ability of the

kidney to concentrate uric acid although no organic changes in the kidney can be demonstrated. Thannhauser, however, built his theory on a very slender experimental foundation and but very few investigations that throw any definite light on these problems have been published in modern literature.

No few investigations into the frequency of the simultaneous appearance of gout and clinically observable organic kidney disease have been published.

Among the more recent investigations Hench, Vanzant, and Nom-bard (184) found "renal complications" in 26 of 100 gouty patients, of whom 14 suffered from renal insufficiency to varying degrees. Williamson (441) examined 116 gouty patients and found albuminuria in 52, of whom 22 had hypertonia as well. Of Schnitker and Richter's (380) 55 gouty patients 5 were found to have uremia, 15 nephrosclerosis, 2 chronic hemorrhagic nephritis, and a further 16 albuminuria only. Bröchner-Mortensen found signs of Bright's disease in 11 out of 58 patients.

The methods evolved during the last few decades for the evaluation of the renal function were, however, but very little used for the investigation of these patients, and very few investigations into the postulated partial functional decrease in the excretion of uric acid have been published.

Endogenous excretion A very large number of authors have investigated the content of uric acid in urine alone.

As has already been mentioned, Garrod (140), in his comprehensive investigations of gouty patients found that they excreted less uric acid than normal persons, which is presumably due to the fact that the diet of gouty patients generally contains less purine. Corresponding results were published later by others, but, after the division of uric acid excretion into endogenous and exogenous, a number of low normal or subnormal endogenous excretions have certainly been found in gouty patients (45, 327, 420), though values varying from the normal are not generally found (36, 38, 60, 110, 148, 179, 219, 280, 432, 433).

The investigation over a long period of 20 persons, the uric acid metabolism of whom might be presumed to be normal, carried out by Bröchner Mortensen (34, 36) showed an average endogenous excre-

tion of from 269 to 532 mg a day (mean figure 374 mg), an average excretion of from 297 to 680 mg a day (mean figure 414 mg) was obtained for 11 gouty patients

Several of the older authors (41, 45, 190, 267, 324) emphasized that the excretion of uric acid varied greatly from day to day for gouty patients, and that a period of especially low uric acid excretion (the anacritical stage) occurred immediately before an acute attack, after a greatly increased excretion during the attack (Harnsaureflut) a new period occurred with decreased excretion (postcritical depression stage) A critical review of these older works, and subsequent investigations show, however, that when due allowance is made for diet, medicaments, and variations in the urine volume, these fluctuations in the excretion of uric acid are very inconstant (36, 409)

Some authors have carried out simultaneous determination of the uric acid in blood and in urine, with a view to the possibility of showing a deviation from the normal excretion of uric acid Steinitz (403) calculated the ratio between the uric acid in the blood (g per liter) and the square root of the uric acid excreted in 24 hours (g), and found values around 0.043 for normal persons, the values for gouty patients being higher (average 0.071) Similar values were published later by Chabanier and Lobo-Onell (60) Gottlieb (149) determined the ratio between the uric acid in the blood and that in the urine after dry diet for 24 hours and found values around 20 for normal persons but considerably lower values for gouty patients

Mathiesen (273) found low normal and subnormal values for the uric acid clearance of a few gouty patients

Brøchner-Mortensen (36, 38) carried out a series of comparative determinations of the uric acid clearance, urea clearance, and creatinine clearance in 20 gouty patients

Definite organic kidney disease was found in 4 of the patients, chronic hemorrhagic nephritis in 2, and nephrosclerosis in 2 Measured by the creatinine and (or) the urea clearance, the renal function was normal in 1 of these patients, and lowered to from 38 to 53 per cent in 3 On the other hand, the values for uric acid clearance were normal (69, 132, 93, and 99 per cent of the normal mean value) The investigation revealed diminished renal function with no other sign of organic kidney disease in 3 of the patients The mean values for uric acid clearance were 42, 41, and 48 per cent

of the normal mean figure, i.e. just below the lowest normal limit, whilst the mean values for the creatine and urea clearance were, respectively, 47, 44, 55, and 47, 37, 63 per cent of the normal mean figure. The values for the creatinine, urea, and uric acid clearance of the remaining 13 of the 20 patients were within the range of variations found in normal subjects.

The total result showed that the uric acid clearance was within the normal variation limit for 17 of the 20 gouty patients and that it was in no case definitely decreased in proportion to the creatinine and urea clearance. The ratio between the concentration indices of the various substances also showed normal values on the whole. The investigation of uric acid clearance without administration of purines thus gives no support to Thannhauser's theory of partial functional decrease, and gives no information of any practical value for the diagnosis of gout.

Repeated investigations of the uric acid clearance of individual patients showed no definite relation between the values found and the acute attack observed during the same period.

Exogenous excretion While no definite difference is found as a rule between the uric acid excretion of normal subjects and that of gouty patients, a great number of investigations seem to show that the exogenous uric acid excretion of the latter is less and that the duration is longer. The investigations carried out by the various authors have, however, given very varying results. This is partly due to different experimental conditions and often to defective analytical technic, and partly to disregard of the influence of variations in the water output on the excretion of uric acid. Finally, the frequent occurrence of fairly considerable variations in the endogenous excretion of uric acid makes the estimation of the endogenous excretion difficult.

No, or at any rate very slight, variations in the uric acid in the blood and in the urine are found as a rule after peroral administration of uric acid. Sivén (392) and later other authors (261, 414, 447) showed that uric acid is decomposed by bacterium coli and other intestinal bacteria both in vivo and in vitro.

Since higher nucleoprotein derivatives are also attacked to a greater or lesser extent by intestinal flora, some of the purine rings being broken, only a fraction of the calculated amount of exogenous uric acid will be recovered from the urine after the peroral administration.

of nucleoproteins or their various derivatives. Sometimes about half of the amount calculated is recovered in normal subjects (29, 54, 389), but there are generally very considerable variations. Frank and Schittenhelm (129) thus recovered from 7 to 41 per cent of the amount calculated.

The exogenous excretion after peroral administration is often less and of longer duration for gouty patients than for normal persons (45, 162, 164, 250, 251, 280, 420, 421), it has, however, in course of time proved to be very difficult to lay down fixed criteria for normal and for pathological excretion.

Ljungdahl (250, 251) found no certain difference between the absolute exogenous excretion of normal subjects and that of gouty patients, the individual variations being very considerable for both groups. On the other hand, the exogenous excretion of gouty patients seems to last somewhat longer. In contradiction to this, McClure and Pratt (280) found, by comparing their investigations with those already published, that 88 per cent of 32 gouty patients excreted less than 20 per cent of the amount administered, while 89 per cent of 35 normal persons excreted over 20 per cent. The average figures for the two groups were 20 and 38 per cent, respectively. On the other hand, no certain difference was found between the two groups as regards the duration of the exogenous excretions. Subsequent investigators also find results that vary greatly and contradict one another. Consequently the investigation is of very slight importance as an aid to diagnosis.

To avoid the highly varying individual influence of the intestinal flora and of the resorption taken as a whole, uric acid was for a time administered parenterally for diagnostic purposes.

The quantity recovered in the urine after intravenous injection of uric acid or purine compounds is considerably greater than after peroral administration. A great many investigators have thus found values in normal subjects that amount to even 100 per cent of the amount administered (63, 90, 164, 411, 416, 421).

Others, however, find greater variations with larger numbers of subjects. Grisbach (155) recovered only from 27 to 66 per cent from normal subjects, Schittenhelm and Harpuder (374, 375) from 18 to 79 per cent, Thannhauser and Weinschenk (417) from 45 to 137 per

cent, Koehler (223) from 23 to 69 per cent, Fohn, Berglund, and Derick (110) from 30 to 90 per cent, and Heydkamp (187) from 90 to 178 per cent. Somewhat lower values are generally found in investigations of gouty patients, and the exogenous excretion often lasts a little longer, but the variation for both groups is still so considerable that the diagnostic value of the investigation will often be problematic.

As in the case of the results obtained by peroral administration, the variations in the endogenous values make exact determination of the size of the exogenous excretion very difficult.

Thannhauser and Hemke (415) suggested that the inability of gouty patients to concentrate uric acid to more than about 50 mg per cent in the urine was the principal reason for the lower exogenous excretion often found for these patients. Vollmond (432, 433), too, found that the ability of gouty patients to concentrate uric acid was deficient, but contemporary (252) and many later investigations show that many cases display no deviation from the results obtained from normal subjects. This is particularly clear when due consideration is paid to the variations in the water output.

Most investigations of exogenous excretion are carried out on the 24-hour urine. The details of the variation in the water output during this long period are not observed, the uric acid therefore being excreted in a urine volume that is sometimes over and sometimes under the augmentation limit. Hence, the details of the variation of the excretion of uric acid also escape observation. Furthermore, even if the variations in the urine volume were considered, the fact remains that the excretion of uric acid varies considerably during the course of the day, and is somewhat constant in the forenoon only.

Brøchner Mortensen (34, 36, 38) therefore carried out a series of peroral and intravenous administrations to normal persons and gouty patients, in which the variations in the uric acid clearance were followed by frequent determinations during several short periods of from 30 to 60 minutes. The investigations were carried out in the forenoon, when the uric acid clearance is liable to the least spontaneous variations. As has been stated above, an increase in uric acid clearance of from 60 to 180 per cent (Fig. III) was observed after administration to normal subjects who had been kept on a purine free

or ordinary mixed diet for some days before the investigation. No variation or, at the most, an increase of up to 50 per cent resulted from

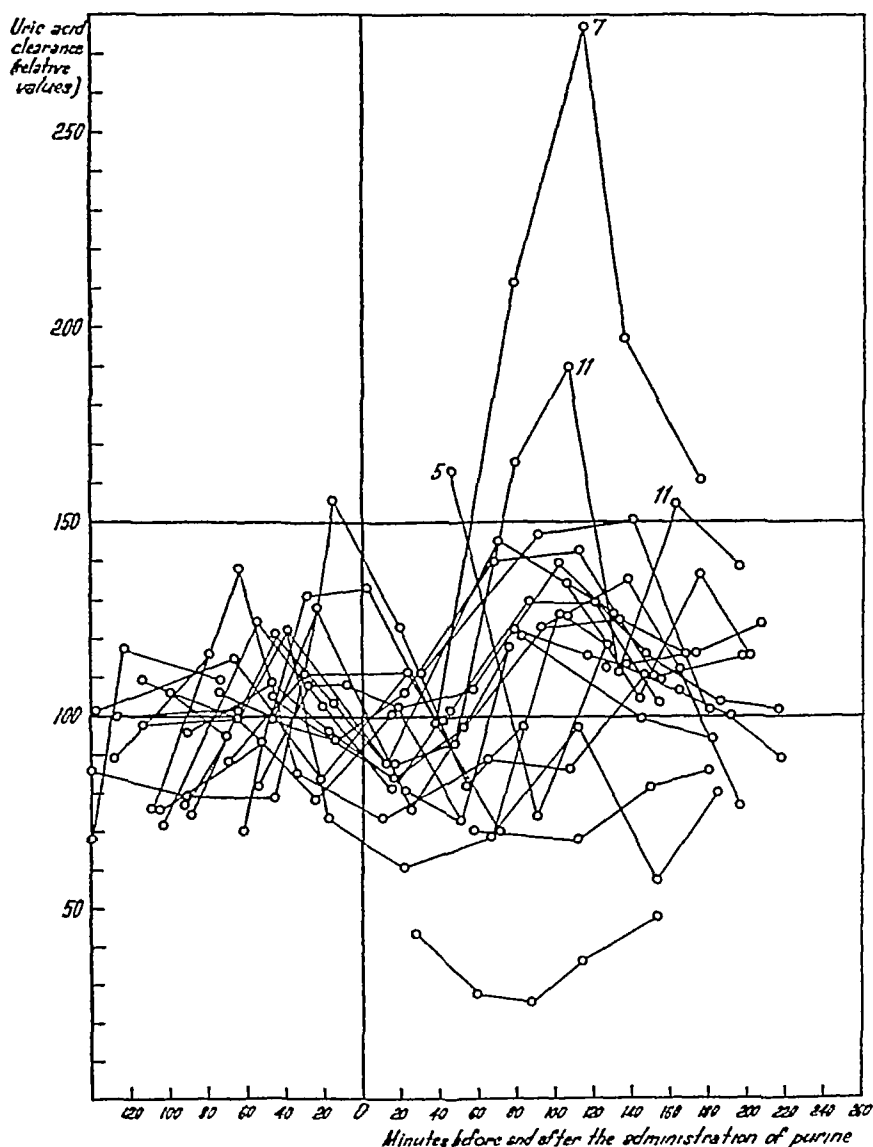


FIG VI URIC ACID CLEARANCE IN GOUTY PATIENTS BEFORE AND AFTER ADMINISTRATION OF PURINE

The values are the percentage of the individual mean value determined before administration

17 administrations to 14 gouty patients, though an increase in the clearance of two further patients was of the same magnitude as that of normal persons (90 and 180 per cent) (Fig VI)

Investigation of the variations in uric acid clearance occurring after administration thus shows features that are not constant enough for any diagnostic significance to be attached to the results. The experiments show, however, with certainty that the excretion of exogenous uric acid by the majority of the gouty patients deviates to a more or less pronounced extent from that of normal persons.

URIC ACID IN THE BLOOD AND URINE IN BRIGHT'S DISEASE

Uric acid in blood

In about the middle of the previous century Garrod (140) demonstrated with his thread test an increase of uric acid in the blood of patients with uremia, but it was not until the close of the century that this observation was confirmed by more exact chemical methods, particularly by v Jaksch (203, 204).

After the appearance of Folin's colorimetric analytical methods in 1912, the question was made the subject of more extensive clinical investigations.

Folin and Denis (113, 114, 115) carried out a series of comparative investigations of the content of uric acid and urea in the blood and found that an increase in the amount of uric acid, without the simultaneous retention of urea, only occurred in gouty patients, whereas patients with Bright's disease and retention of urea gave values that were sometimes normal, sometimes increased.

Later on, however, a long series of reports were published, especially in America, that seemed to show that uric acid retention is an early and important sign of incipient renal insufficiency.

Myers and Fine (311), Myers and Lough (313), Chace and Myers, (61), Myers, Fine, and Lough (312) thus often found hyperuricemia in patients with kidney disease without retention of urea and creatinine though, on the other hand, retention of urea was always accompanied by hyperuricemia.

Krauss (226) found from repeated examination of patients with acute and chronic hemorrhagic nephritis that the uric acid was often retained before the other non protein nitrogen substances, and moreover that the hyperuricemia lasted longer during recovery than the retention of urea. An increase to over 10 mg per cent uric acid in the blood was considered to be a sure sign that the course of the kidney disease would be rapid and fatal.

Similar observations have been reported by many other authors (11, 83, 96, 142, 165, 205, 231, 246, 268, 271, 284, 290, 291, 309, 326, 429, 430, 434)

A number of these even thought they were able to assert that the determination of uric acid was of more importance in the examination of patients with Bright's disease than the determination of urea

In this connection Krauss (226) asserted that "die bequem auszuführende \bar{U} -bestimmung im Blut kann demnach bei der Beurteilung von Nierenerkrankungen die Bestimmung der übrigen N-komponenten meistens erübrigen," and, as late as 1934, somewhat the same was said by Lichwitz (246)

This view has only been adopted to a very slight extent in clinical practice The reasons are, presumably, partly the generally prevalent uncertainty as to the methods of analysis, and partly that hyperuricemia can be found in many diseases without renal affection, in particular in gout, cardiac failure, diseases of the liver, diabetes mellitus, various diseases of the blood, febrile diseases and poisoning with various substances

It has, furthermore, been asserted from various quarters that, though retention of both urea and uric acid is certainly found in pronounced renal insufficiency, there is generally no proportion between the quantities of these two substances, and that the amount of urea retained is the best indication of the course of the disease Feinblatt (96) thus gives an account of two patients with uremia, in the blood of each of whom about 400 mg per cent urea was found, but 35.5 and 50 mg per cent uric acid, respectively Several investigators of uremia observed first retentions of uric acid and urea that followed a parallel course and later on, in spite of increasing retention of urea, constant or even falling values for uric acid The cause of this was, presumably, partly a compensatory increase in the excretion through the bile and through the gastric and intestinal secretions with consequent destruction by the action of the intestinal bacteria, through which the possibility of reabsorption was decreased

Finally, it must be emphasized that Holbrook and Haskins (192) in their investigations of 126 patients with Bright's disease found retention of uric acid in no case earlier than the retention of urea Woods (444), too, often found retention of urea but not of uric acid in patients with Bright's disease No tendency was generally seen in

Bröchner-Mortensen's (35) investigations with the potassium ferricyanide method towards the retention of uric acid in the blood before that of urea, relatively much greater retention of urea than of uric acid being observed in severe cases of renal insufficiency (Fig VII)

A number of authors have compared the content of uric acid in the blood with the results of various renal function tests

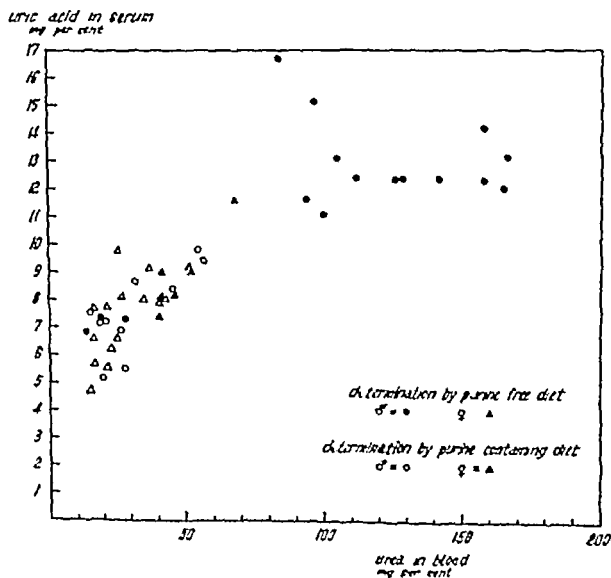


FIG. VII RATIO OF URIC ACID TO UREA IN THE BLOOD OF PATIENTS WITH BRIGHT'S DISEASE

Myers, Fine, and Lough (312) found hyperuricemia in a number of patients with no remarkable diminution in the excretion of phenol-sulphonephthalein

Chabanier and Lobo-Onell (60) sometimes found normal uric acid values in the blood in spite of a pronounced increase in Ambard's constant. There was no proportion between the content of uric acid in the serum and Ambard's constant in the patients with hyperuricemia

Johnston (208) made simultaneous determinations of the uric acid in the blood and the urea clearance, and found only very poor agreement in the variations. In many of the cases in which the clearance was below 20 cc a minute normal uric acid values were found, and Brøchner-Mortensen (35) found very similar results on comparing the content of uric acid in the blood (potassium ferricyanide method) with the clearance of urea and creatinine.

Uric acid in urine

Determination of the uric acid in the urine alone gives, as a rule, no reliable indication of the functional abilities of the kidney. The amount excreted through the kidneys does not decrease to any definitely measurable degree until there is a very considerable decrease in the renal function (35).

Somewhat more pronounced changes are seen when the content of uric acid in the blood is compared with that in the urine.

Watanabe (434) investigated the relation between the uric acid in the blood (expressed in mg per cent) and the amount of uric acid excreted in an hour (expressed in mg) and found from 0.10 to 0.30 for normal subjects and up to 2.2 for patients with renal insufficiency. Steinitz (403) calculated the ratio between the uric acid in the blood (g per liter) and the square root of the uric acid excreted in 24 hours (g), and found values between 0.037 and 0.051 for normal persons and considerably higher values for patients with Bright's disease. Similar investigations were carried out with corresponding results by Highley and Upham (188), Chabanier and Lobo-Onell (60), et al.

Gottlieb (149) calculated the ratio between the concentration of uric acid in urine and in blood after a dry diet for one day and found values around 20 for normal subjects and considerably lower values for patients with renal insufficiency.

Gardstam (139) investigated the relation between the excretion of uric acid and that of creatinine by a calculation of the "Exkretionsprocentsatz" (uric acid clearance expressed in percentage of creatinine clearance) and 'Exkretionsindex' (the amount of uric acid excreted per minute expressed in percentage of creatinine clearance). Very constant values were found for normal persons and patients with simple toxic and orthostatic albuminuria, 8.5 ± 2.7 and 0.31 ± 0.06 , respectively, while, on the other hand, considerable increases were

often found in patients with acute and chronic hemorrhagic nephritis, pyelonephritis, and nephrosclerosis. An increase in the "Exkretionsindex" was assumed to be a sure early sign of renal insufficiency. A critical review of Gårdstam's (139) and Brøchner-Mortensen's (35) studies shows, however, that lowered creatinine and, especially, urea clearance is very often found in spite of normal values for the "Exkretionsindex."

Berglund and Frisk (24) calculated the so-called "Elimination-index," the ratio between the amount of uric acid excreted in one hour

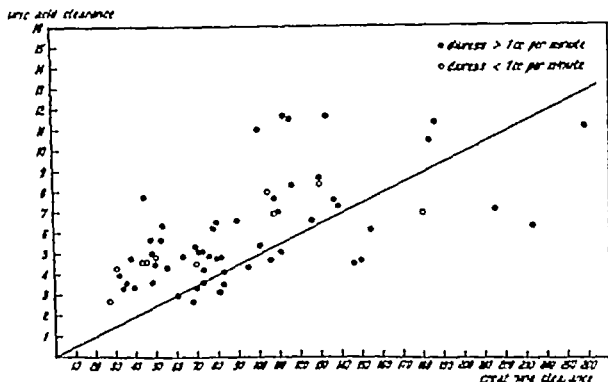


FIG VIII RATIO OF URIC ACID CLEARANCE TO CREATININE CLEARANCE FOR PATIENTS WITH BRIGHT'S DISEASE

The line inserted shows the mean value of the data for normal persons.

(mg) and the content of uric acid in the blood (mg per cent), and found values between 5.1 and 19.1 (average 11.4) for normal subjects. The values for patients with kidney disease decreased proportionately with the creatinine clearance (coefficient of correlation $+0.80 \pm 0.04$). No certain tendency to a relative increase in the elimination index with a decreasing creatinine clearance was found.

Brøchner-Mortensen (35) investigated a series of patients with various kidney diseases and found that the maximum uric acid clearance varied between 2.6 and 15.8 and that the standard uric acid clearance varied between 0.9 and 8.3, the corresponding values for normal persons being from 3.5 to 10.2 and from 3.7 to 11.7. On the

whole, the uric acid clearance decreased simultaneously with the diminution in the urea and creatinine clearance, especially when the deviations from the normal were very slight (Figs VIII and IX). The reduction is, however, not proportional, for the uric acid clearance will often be relatively better maintained than that of creatinine and of urea, in many cases it may be completely normal, in spite of a considerable decrease in the urea and creatinine clearance, presumably because of a decrease in the reabsorption of uric acid in the tubules. It is, however, very doubtful whether any diagnostic or prognostic

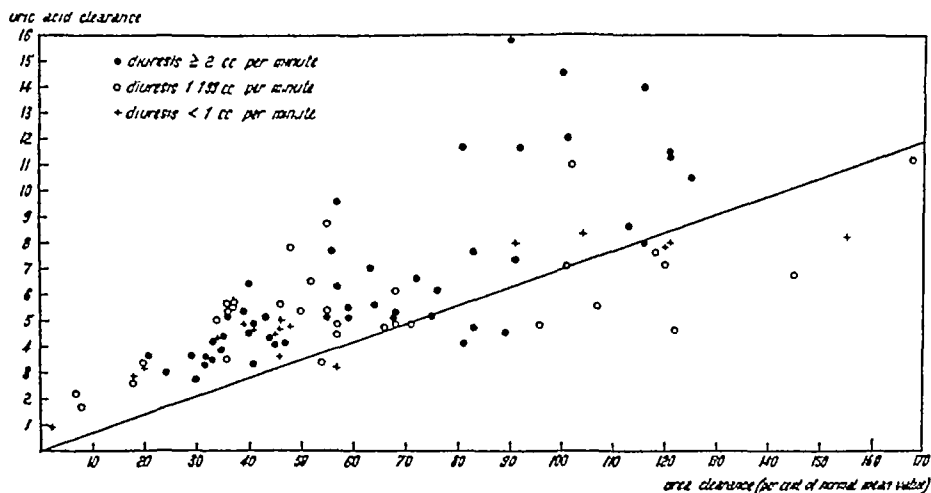


FIG IX RATIO OF URIC ACID CLEARANCE TO UREA CLEARANCE FOR PATIENTS WITH BRIGHT'S DISEASE

The uric acid clearance is calculated by the formula for the maximum clearance with a water output of >1 cc per min, and by the formula for the standard clearance with a water output of <1 cc per min. The urea clearance is shown as the percentage of the normal mean figure ($C_m = 75$, $C_s = 56$). The value is calculated by the formula for the maximum clearance with a water output of ≥ 2 cc per min, and by the formula for standard clearance with a water output of <2 cc per min.

The line inserted shows the mean value of the data for normal persons

significance can be attributed, as it has been by Gardstam, to the variations in the ratio between the clearance of the various substances, since quite similar variations can be found in patients whose kidneys are, to all appearances, intact

URIC ACID IN THE BLOOD AND URINE IN VARIOUS OTHER DISEASES

A large number of observations have been published dealing with changes in the content of uric acid in the blood and in the urine in various pathological conditions besides gout and Bright's disease

Hyperuricemia and increased endogenous excretion of uric acid, caused by increased decay of the tissue, are often seen in patients with *acute febrile diseases*, pneumonia, sepsis, typhoid, erysipelas, scarlatina, and tuberculosis (89, 96, 130, 203, 204, 205, 221, 222, 226, 308, 379, 388, 429, 439). It has very often been observed that such diseases brought on attacks of gout, and the same is seen after *poisoning* by carbon monoxide, ammonia, veronal, methyl-alcohol, etc. (82, 226, 333, 379), in some cases partly in consequence of renal damage.

Several investigators have announced that they have found a decrease in the content of uric acid in the blood and an increased excretion during treatment with *X-rays* and *radium*. The changes, however, are very uncertain and inconstant (29, 97, 159, 216, 220, 319, 400).

A low concentration of uric acid in the blood is often found in *acute and chronic polyarthritis* (66, 211, 221, 222, 326, 402, 432, 433), and a high excretion (423), caused, possibly, by salicylate treatment.

An increase of uric acid is generally found in the blood and urine of patients with *leukemia* (45, 126, 212, 221, 222, 226, 266, 340, 379), and in a number of cases the simultaneous occurrence of leukemia and gout has been described (12, 46, 57, 91, 146, 167, 320, 322, 341, 385, 425). It is, however, impossible to decide from existing publications whether the simultaneous occurrence of the two diseases is more frequent than might be expected from fortuitous coincidence.

Very varying values are found in the blood and urine of patients with *pernicious anemia* (144, 145, 213, 344, 345, 346, 419), though normal or slightly subnormal values have been found in most of the untreated cases. A moderate increase in the uric acid in the blood and a pronounced increase in the amount of endogenous uric acid excreted are generally seen during treatment with liver extract almost simultaneously with an increase in the number of reticulocytes. According to Riddle (345) this is even so constant that on a rise of 1,000,000 per c. mm. in the erythrocyte count a simultaneous increase of about 10 g. in the excretion of uric acid is found. In many cases attacks of gout have been observed simultaneously with this intensive formation of endogenous uric acid (185, 386, 397).

Corresponding increases in the amount of endogenous uric acid have been observed during regeneration of the blood after *hemor*

rhage (224) or in *hemolytic jaundice* (239) Hyperuricemia is also often found in patients with *polycythemia* (199, 379), it is frequently noted as a general clinical experience that gout often occurs in these patients Few of these cases have, however, been published (36, 38, 282, 341, 380, 436)

Increased values for the uric acid in the blood and in the urine are not infrequently seen in patients with *diseases of the liver and the biliary system* (27, 33, 70, 71, 96, 135, 340, 372, 379, 419) The cause is partly decreased excretion of uric acid through the biliary system and partly increased formation of endogenous uric acid resulting from decay of the nuclein-rich parenchyma Hyperuricemia is, however, not found constantly, and often does not occur in the severest cases of liver damage (70, 334, 372, 379)

The relation between hepatogenous hyperuricemia and the occurrence of gout has often been discussed, but has not been fully investigated More or less pronounced changes are not infrequently found on examination of the liver function in gouty patients (36, 71, 349)

Hyperuricemia is often found in patients with *arterial hypertension* and *severe cardiac decompensation*, in certain cases with no demonstrable sign of decrease in the renal function (99, 205, 226, 231, 290, 379)

It is, furthermore, observed in patients with severe *diabetes mellitus*, particularly in cases with acidosis (226, 294, 310, 338, 379) Normal values are generally found for the uric acid in the blood of diabetics without acidosis, the values in a few cases being around the upper normal limit (170)

A considerable endogenous excretion of uric acid (about 1 g a day) is found in *acromegaly* (94, 227, 377, 412)

Hyperuricemia, generally moderate, is sometimes found in *chronic eczema*, *psoriasis*, *urticaria*, and allied diseases (26, 74, 96, 234, 270, 368, 369, 448)

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ACUTE HEMOLYTIC ANEMIA (ACQUIRED HEMOLYTIC ICTERUS, ACUTE TYPE)¹

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INTRODUCTION

The recent observation of 4 cases of acute hemolytic anemia responding dramatically to splenectomy proved of especial interest when an active hemolysin was repeatedly demonstrated in the serum of two of them (65). Hemolytic icterus as an entity distinct from the obstructive type was first definitely described in 1900 by Minkowski (234) and separated into congenital and acquired types by Chauffard (43), Widal (364) and their collaborators. Chauffard and Troisier (47) furthermore described a third ("hemolysinic") group, which was characterized by the presence of iso-hemolysins in the serum. Numerous studies on fragility, reticulocytosis, auto-agglutination, and hemolysins were carried out by Chauffard (43), Widal (361, 362), and their pupils but were apparently abruptly terminated with the beginning of the World War in 1914. In the following decade, many of the teachings of the French school were apparently forgotten, for the report by Lederer (197) of 3 cases of acute hemolytic anemia in 1925 was hailed as that of a new disease entity. With rare exceptions, the numerous authors reporting cases of this acute form of acquired hemolytic icterus have ignored the older literature and have usually designated the condition as "Lederer's anemia."

The finding of hemolysins in our cases led to various studies relating to the pathogenesis of the hemolytic syndromes (66). The conclusion was reached that these conditions (hemoglobinurias, most types of hemolytic icterus or anemia) often differed more in degree than otherwise, and might be brought about by the action of hemolysins of various types and "dosages." An outstanding feature of the blood picture in both the clinical and experimental cases was the tendency of the red cells to assume a spherical shape, and thus to

become abnormally fragile to hypotonic solutions of sodium chloride. It was concluded that spherocytosis (and abnormal saline fragility) were dependent upon the activity of various types of hemolysins upon circulating erythrocytes, and not upon an abnormal type of erythrocyte production within the marrow.

Many of the teachings of Chauffard, Widal, Troisier (332) and others of the French school from 1907-1915 seem refreshingly new when compared with some of the more recent literature. Thus, with respect to classification of hemolytic anemia (icterus), the old terminology of congenital and acquired types, amongst which acute, sub-acute, and chronic forms are discriminated, seems more rational than the present rather confusing picture. Although acute hemolytic anemia (the acute type of acquired hemolytic icterus) is probably not a separate disease warranting an eponym, it is nevertheless of exceptional interest since it permits much insight into the pathological physiology of the hemolytic states. The dramatic response of our cases to splenectomy, when transfusions were ineffective, immediately implicates the spleen as perhaps the central organ of abnormal hemolytic activity.

The report which follows is based upon clinical and experimental studies carried out in connection with 4 cases of acute hemolytic anemia together with a critical review of the literature. Although these studies have led us rather far afield into immunology and the physiological pathology of the spleen, they have, however, served to unify for us the usually widely separated hemolytic states and to demonstrate such common denominators amongst them as spherocytosis and hemolysin activity. In these respects, the study of this rather rare condition has proved of great profit.

CRITICAL REVIEW OF LITERATURE

A Historical

Although sporadic reports of cases of anemia associated with icterus differing in some respects from the progressive pernicious anemia of Biermer had been published for several years, the knowledge and the differentiation of hemolytic anemia from actual disease of the liver may be said to have begun with Hayem in 1898 (150) and Minowski (234) in 1900. At the 18th Congress of Internal Medicine

held in 1900, the latter author reported a case of chronic hereditary acholuric icterus associated with urobilinuria and splenomegaly. Because of the finding at post-mortem examination of marked siderosis of various organs, Minkowski hypothecated an abnormal destruction of red blood cells due, he felt, to a primary lesion of the spleen. This report was soon followed by a number of others citing similar cases, but no significant advance took place until the publications of Chauffard (43-50). This investigator in 1907 discovered (43) that the red cells in this congenital disorder were unusually fragile as evidenced by their behavior towards hypotonic solutions of sodium chloride. In publications which followed (44-50), Chauffard successively standardized the fragility test, described the reticulated red blood cells (reticulocytes) and demonstrated their greatly increased number in congenital hemolytic icterus, and drew attention to the microcytic character of the blood picture. Chauffard's publications are models of lucidity and thoroughness, and portray the best of clinical investigation. During the same period—from about 1908 to 1914, Widal, Abram, and Brulé (361-367) published a series of observations on a type of hemolytic icterus which was not congenital in type, and which appeared either gradually or suddenly in the course of various illnesses, or as a disease *suu generis*. They called these cases "acquired hemolytic icterus," and pointed out the lack of an hereditary factor, the generally slight alteration in fragility test as compared with the congenital type, the presence of marked reticulocytosis and of autoagglutination of the red cells. These observations were confirmed by many others and thus two types of hemolytic icterus or anemia were discriminated: the congenital type of Chauffard and Minkowski and the acquired type of Hayem and Widal. Chauffard with his co-workers Troisier and Vincent in 1908 and 1909 reported two cases of hemolytic icterus differing from both of these types by the presence in the serum of an hemolysin capable of hemolyzing human red cells (isohemolysin). They called these cases "hemolysinic icterus." The second case was extremely acute in its course and associated with hemoglobinuria. Chauffard and Vincent effectively pointed out the physiologic relationships existing between acute paroxysmal hemoglobinuria on the one hand and congenital hemolytic icterus on the other.

Widal, Abrami, and Brulé differentiated chronic, subacute, and acute cases in their series of acquired examples Türk (337), in his comprehensive series of lectures published in text-book form (1912), stated that a group of cases of hemolytic anemia resembled pernicious anemia rather closely, but could be distinguished from it by various clinical and hematological criteria In this group was a "Type S" (named after a patient with initial S) characterized by subacute course, tendency to relapse, splenomegaly, and pernicious anemia-like blood picture He finally conceded the similarity of this group with the acquired hemolytic icterus of the French workers Also under the heading of Group IV he described as "acute hemolytic anemia" a case characterized by rapidly progressive down-hill course, fever, splenomegaly, urobilinuria, severe anemia, leukocytosis, macro and microcytosis, polychromatophilia, and fatal termination

In 1911 Micheli (231) reported, for the first time, the beneficial effect of splenectomy in a case of acquired hemolytic icterus In 1912 and 1913 Banti (10) described two cases of "hemolytic splenomegaly" characterized by anemia, acholuric icterus, and splenomegaly, and responding dramatically to splenectomy Banti's detailed reports dealt with the fragility test, the mode of action of the hemolytic substance, the hemolytic activity of splenic extract, and the effects of splenectomy He concluded that the spleen, through the medium of cytolytins produced by its large content of mesenchymal cells was the principal organ of hemolysis and that its removal in the "primary" cases of splenopathy with icterus was rational Aside from the routine treatment with iron and arsenic, this therapeutic procedure was the first specific type of therapy which had thus far been introduced

Antonelli (8) (1913) in a comprehensive article on hemolytic icterus, criticized Banti's concept of "hemolytic splenomegaly" and pointed out that this condition differed in no wise from that of the acquired hemolytic icterus of the French school After a thorough historical review of the entire subject, he described a case of "acquired hemolytic icterus with anemia of the pernicious type" This was of the subacute variety and responded dramatically to splenectomy Antonelli concluded that the extraordinary effect of splenectomy demonstrated that splenopathy was a pathogenic factor of great importance in the

hemolytic process but could not be represented as the prime or only cause of the disturbance

Roth (300) in 1913 reported an acute case of hemolytic icterus which showed both an autohemolysin and an autoagglutinin in the blood serum. He criticized Chauffard and Vincent's (50) conception of an "hemolytic" type of icterus and postulated an abnormality of the red cells. Because of its rapidity of course, and the presence of free HCl in the gastric juice, he regarded his case as an atypical example of pernicious anemia. The patient died.

Eppinger (102) and other members of the Vienna school soon became convinced of the dramatic effect of splenectomy in cases of the acquired type of hemolytic jaundice and the first general article on the effects of splenectomy in diseases of the blood was published by Eppinger and Ranzi (103) (1914). From Vienna also came the detailed description of a case of acute hemolytic anemia (Nobel and Steinebach (250), (1914) responding to splenectomy and in which *dense, hemoglobin-laden microcytes* were conspicuous. That these were typical spherocytes is brought out in an illustration of the blood picture from this case in Eppinger's book on the Hepato-Lienal Diseases (102) (p. 206). In this volume, which in many respects was greatly ahead of its time, the various types of hemolytic icterus are well described and differentiated. The congenital and acquired types are demarcated, and among the latter are distinguished secondary and cryptogenetic or primary types. The blood picture in both the congenital and acquired types is described as entirely similar, and the erythrocyte fragility as abnormal in both instances. In 1919, Naegeli (245) first used the word "spherocyte" for the "dense, hemoglobin-laden microcytes" above referred to and thereupon laid down the dictum that this cell was pathognomonic of congenital hemolytic icterus and was the result of abnormal erythrocyte formation in the bone-marrow. Like many other statements made by Naegeli, this was soon accepted as gospel and the concepts that the spherocyte and increased fragility were (a) pathognomonic of congenital hemolytic jaundice and (b) formed in the bone-marrow became deeply ensconced. Simultaneously, various authorities began to doubt the validity of the concept regarding the acquired form of hemolytic jaundice. As stated by Meulengracht (229) (1922) "under

this designation, many separate disease syndromes are collected." Gradually the diagnosis of acquired hemolytic icterus was cast into question.

In 1925, Lederer (192) published as a "new" syndrome under the title of "Form of Acute Hemolytic Anemia—Probably of Infectious Origin" a group of 3 cases characterized by acute onset, rapid course, fever, acholuric icterus, splenomegaly, marked anemia, and leukocytosis, in which the dramatic response to transfusion of blood was emphasized. These cases were identical with the previously described cases of Chauffard and Vincent, Nobel and Steinebach and several others. Following Lederer's paper, similar cases were soon reported, and by 1930, when Lederer (198) reported 3 further cases, the concept that a new type of anemia was present—"Lederer's anemia"—seemed deeply rooted. In none of these articles was the older literature of acquired hemolytic icterus discussed. For example, the several publications on "hemolysin" icterus of a previous generation were almost completely neglected and forgotten. Many investigators were well acquainted with Widal's phenomenon of auto agglutination in cases of acquired hemolytic icterus and, at least until 1914, tests were often made for the presence of autohemolysins, isohemolysins, and heterohemolysins in the serum. These serological factors were not mentioned in Lederer's articles, and search for them was in no recent case reported until the article of Davidson (69) in 1932 in which marked spontaneous auto agglutination is reported in Case 9 (also reported by Troisier and Cattani (334) and Patterson and Smith (264)). When Giordano and Blum (121) recently found autoagglutination in Case 2 of their series, they stated that "the reported occurrence of spontaneous autohemagglutination in the course of acute hemolytic anemia is observed for the first time." That hemolysins might be present in the serum was not "rediscovered" (cf P. D. White (360)) until the present authors' recent publication (65).

Lederer's chief contributions were in the rediscovery of a syndrome which had been lost sight of for more than a decade and in his demonstration of the great therapeutic efficacy of transfusions of blood. We feel, however, that the use of the eponymic "Lederer's anemia" is unwarranted because of the undoubted priority of previous descriptions. At this point, criticism may be made that the French and

Italians described cases of hemolytic "icterus" and not of hemolytic "anemia." That there cannot be any possible distinction between these terms should immediately be apparent. Icterus due to hemolysis (red cell destruction) must of necessity be associated with anemia, since if icterus is present sufficiently to be noticed, some anemia will almost always be present. Although most French authors use the terms synonymously, Fiessinger, Decourt, and Laur (108) attempt to discriminate between "acute hemolytic anemia" and "acquired hemolytic icterus" by stating that although identical signs are present, icterus is dominant in the cases of acquired hemolytic icterus, whereas anemia is outstanding in the former disease. These arguments seem academic in the extreme and are not borne out in the examination of the case reports of "acquired hemolytic icterus." One must conclude that the use of the term "hemolytic anemia" as against "hemolytic icterus" is simply a matter of taste.

B Cases in literature

About 100 cases of acute and subacute hemolytic anemia have been reported by about 80 different observers since 1907. Any list of these cases must of necessity be inexact since many cases of acute hemolytic anemia are undoubtedly buried under the titles of atypical anemia, progressive pernicious anemia, atypical pernicious anemia, pernicious anemia responding to splenectomy, etc. The following is a partial list of the titles which have been used in describing this group of cases: acute hemolytic anemia—probably of infectious origin—Lederer (197), acute hemolytic febrile or infectious anemia (Lederer (198)) (Altmann (5)), acquired hemolytic icterus with anemia of pernicious type (Antonelli (8)), acute febrile pernicious-like anemia (Antognetti (7), Campanacci (37), Greppi and Semanza (128)), hemolytic splenomegaly (Banti (10)), acute febrile pernicious anemia, acute pernicious anemia, acute febrile anemia, acquired hemolytic icterus, hemolytic icterus (47), Lederer's acute hemolytic anemia, Lederer's anemia, acute anemic syndrome of Lederer, acute hemolytic anemia—Lederer's type, macrocytic hemolytic anemia (68), acute hemolytic anemia, acute anemia, acute grave anemia.

In the consideration of the cases of hemolytic anemia suitable for inclusion in our group of "acute" cases, the following criteria have

been set up (1) history of acute or fairly acute onset usually with gastro intestinal symptoms, rapidly progressive pallor and weakness, (2) signs of marked anemia with definite icterus and splenomegaly, (3) evidences in the blood of severe anemia often described as macrocytic in variety, (4) signs of increased blood formation leukocytosis, young granulocytes, polychromatophilia, reticulocytosis, nucleated red cells, (5) evidence in the body fluids of increased blood destruction such as bilirubinemia, increased urobilin output in the stools and urine, (6) evidence against pernicious anemia such as lack of glossitis, absence of central nervous system phenomena, and presence of free HCl in the gastric juice, (7) lack of demonstrable or obvious cause for the hemolytic process such as bacteria, chemicals, neoplasms, and pregnancy, and finally (8) complete absence of similar disease in the family

In reporting this series of cases, another difficulty lies in the separation of acute cases from those substantially similar cases which are chronic and subject to relapses. Whenever the chronicity of the process has been obvious, these cases have been excluded. It has been impossible, however, nor is it wise, we feel, to differentiate between the acute and the subacute cases. The acute fulminating case is one (cf. Case 3 of our series) in which the course is one of a few days or a week and is not infrequently associated with hemoglobinuria, the subacute case (Cases 1, 2, and 4 of our series) will be found to have a history of 1 to 3 months of gradually increasing fatigue and weakness, with final appearance for medical attention when marked anemia and fever are present.

Many cases of acute hemolytic anemia have been reported in which a definite cause of the condition has been evident. These cases are in many or all respects similar to the "idiopathic" ones in course, symptomatology, and blood picture. Widal, Abrami, and Brulé (363 et seq.) recognized that various etiological factors might be present. They listed malaria, streptococcic and staphylococcic infections, tuberculosis, anaerobic organisms including the bacillus Welchii and anchylostomiasis as possible causes. In recent years, with the growing increase in the use of various drugs, cases have been reported following the use of arsphenamine, neoarsphenamine, acetanilid (318), phenylhydrazine (176), and sulphanilamide (142, 177)

Lead poisoning may also be a cause (9) Numerous cases of hemolytic anemia in association with pregnancy have also been reported (392, 256, 278, 304, 321, 372) and the marked similarity if not complete identity of these cases with the acute (idiopathic) hemolytic anemia has occasionally been mentioned Cases are also not infrequently seen in association with such conditions as Hodgkin's disease (68, 314a), lymphatic leukemia (262a) and carcinomatosis (348) As far as possible, these "symptomatic" cases have been excluded from consideration in this study

The first cases of acute hemolytic anemia were reported by Widai, Abram and Brulé (361 et seq), Chauffard and Troisier (47), Chauffard and Vincent (50), and Teeter (324) Except for Lederer's 6 cases, the largest series of cases are reported by various English writers Davidson (68), Joules and Masterman (174), O'Donaghue and Witts (253), and Parsons and Hawksley (262) These observers each report from 4 to 9 cases in their papers Careful analysis of their cases shows in most instances, however, that a number of different entities are grouped together under the titles of acute hemolytic anemia or macrocytic hemolytic anemia, etc Thus of Davidson's 9 cases, three are Hodgkin's disease in which hemolytic anemia was also present and one was a case of lead poisoning, Case 9 was a chronic case Four cases were fairly typical of the acute idiopathic type described in this paper Of Parsons and Hawksley's widely quoted series of 9 cases reported in 1933 as "the hemolytic (erythronoclastic) anemias of later infancy and childhood" only one (Case 1) was typical, another one (Case 6) being a possible case Case 2 was apparently aplastic anemia, Case 3 "non-leukemic reticulo-endotheliosis," Cases 4 and 5 "nutritional anemia," Case 7 one of acute leukemia, and Cases 8 and 9, which had rickets, showed pallor without icterus, bilirubinemia or urobilinuria The published cases which we believe are acceptable for inclusion in our category of "acute hemolytic anemia" are as follows Aguirre *et al* (2), Altmann (5), Anderson (6a), Antognetti (7), Antonelli (8), Baxter and Everhart (14), Benhamou (17), Brill (30), Calvin (36), Campanacci (37), Castex *et al* (39), Chauffard and Troisier (47), Chauffard and Vincent (50), Chevallier *et al* (51), Christiansen (53), Colarizi (56), Corelli (58), Dalla Volta (62), Dameshek and Schwartz (4 cases), Davidson

(Cases 6, 7, 8, 9) (68), Davidson and Fullerton (69), Decastello (71), Douglas (81), Dunlop and Saunders (87), Eimer (97), Eppinger (102), Fiessinger *et al* (108), Giordano and Blum (2 cases) (121), Goudsmid (2 cases) (124), Greenwald (2 cases) (126), Greppi (127), Greppi and Semanza (2 cases) (128), Hampson and Warner (2 cases) (145), Heilbrun (153), Holst (163), Israëls and Wilkinson (4 cases) (167), Joules and Mastermann (4 cases) (174), Kaiser and Bradford (175), Kühl (189), Landor (190), Lazarus (2 cases) (196), Lederer (6 cases) (197, 198), Lemaire and Portier (199), Livingston and Edwards (211), Lovibond (213), McGavack (216), MacIntosh and Cleland (218), Manfredini (220), Manne and Kuskin (221), Mendels (3 cases) (226), Nixon and Vaughn (248), Nobécourt and Liège (249), Nobel and Steinebach (250), O'Donoghue and Witts (2 cases) (253), Parsons and Hawksley (Case 1) (262), Patterson (263), Payne (265), Pearce (266), Planteydt (273), Rastetter and Murphy (2 cases) (282), Rettanni (287), Reynolds (288), Rosenthal and Corten (296), Roth (300), Schwarz (309), Shackle (311), Stark (316), Steffens (317), Sturpe (319), Tangredi (323), Teeter (324), Tixier (331), Troisier and Catton (334), Türk (337), v Stejskal (343), Weber-Bode (351), Weil *et al* (352), Widal, Abram and Brulé (364), Widal and Weissenbach (369), Williams (371)

C Analysis of Cases

Onset and course Since this paper deals with "acute hemolytic icterus," an acute onset is naturally presupposed. However, in reviewing the literature, it is not easy to discriminate between chronic cases which had probably been "smouldering" for months to years and acute cases in which the condition showed definite and progressive development within a period of a month or less. Even in our own cases, closely observed as they were, the sharp distinction between acute, subacute, and chronic was not always possible. In Case 1 of our series, acute symptoms had been present for about a month, but the patient was a stolid individual and his wife stated that he had been "sallow" for about two years, and that four months previously an attack of "intestinal grippe" was present. In Case 2, acute symptoms had also been present for about a month, but for about two months the patient had felt rather tired, and for eighteen months a

slight "idiopathic" anemia had been present. This patient, furthermore, had had cholecystectomy performed about a year before onset of the present illness, and one wonders about the possible association of this condition with chronicity of the hemolytic process. Case 3 was fulminating, the symptomatology being only of five days' duration, the patient having been entirely well prior to this illness. In this case, however, the presence of spherocytic red cells and abnormal fragility made several observers suspect the possibility of previously unrecognized congenital hemolytic icterus. The past history was, however, completely negative, and investigation of the family showed no evidence of the condition. In Case 4, which was thought by several observers to be a typical example of acute non-congenital hemolytic anemia, the demonstration of spherocytes and of lack of response to transfusions caused the same observers to change their diagnosis to that of chronic (unrecognized) congenital icterus.

In general, however, the present illness was of acute onset beginning usually less than a month before admission to the hospital for treatment. In about 25% of the reported cases, the disease was subacute in type with symptoms having been present for at least a month prior to observation. In several of the cases reported as "acute," the symptoms had been present for three to six months.

The course in the cases which gave a history of at least a few weeks of illness was characterized by progressive increase in weakness and dyspnea with gradual increase in severity of symptoms until finally the patient entered the hospital often in a semi-moribund state. The velocity of course was apparently far more rapid when the patient developed severe symptoms than during their gradual early development. In some of the early cases, after a period of progressive downhill course, there was a more or less sudden turn-about and the patient showed progressive spontaneous improvement. In some, however, death supervened. In the more recently described cases the downhill course was either interrupted by transfusion, liver extract, or splenectomy. In general, the types of course may be arbitrarily listed as follows: (1) acute fulminating—with a history of a week's illness or less—hemoglobinuria often present—high fever as a rule, (2) acute, with a history of about a month's illness with gradual development of symptoms, (3) subacute, with symptoms of a few months' duration.

The chronic, unsuspected case with an acute exacerbation frequently cannot be separated from these types

Symptoms No specific symptomatology is present. In general the symptoms are (1) those of an acute febrile illness, (2) those relating to the gastro-intestinal tract, (3) and those of anemia. In many cases, the passage of dark-colored urine has been noted. In Case 1 of our series, subacute in type, the symptoms were principally those of anemia: progressive weakness, increasing dyspnea, and edema of the ankles. At times the vomiting of "bilious" material had been noticed and highly colored urine had been passed for about a month. In Case 2, also subacute in onset, the symptoms were those of anemia: fatigue, dyspnea, progressive pallor. Later there was vomiting with the development of malaise and fever. In Case 3, acute in type, there was malaise, "dopiness," and fever prior to the development of symptoms of anemia and the passage of dark-colored urine.

The symptoms suggestive of an acute febrile illness are well described in Lederer's cases (197, 198): sudden weakness often becoming extreme, malaise, headache, restlessness and irritability (in infants), pain in the back and extremities, "grippe" with fever of 101-102°F for two weeks (Case 3), and anorexia. The symptoms relating to the gastro-intestinal tract, besides that of anorexia, are nausea and vomiting, diarrhoea, pain in the region of the spleen (Brill (30)), sensation of weight in the epigastrium (Greppi (127), Heilbrun (153), McGavack (216)), and vague abdominal pain (Nobécourt and Liège (249)). Diarrhoea seems to be more common in infancy. The symptoms of anemia are as described above: weakness, fatigue, dyspnea, palpitation of the heart, and edema of the ankles. A peculiar yellowish color, even definite jaundice was noted by some patients. A further hint from the history of a hemolytic type of anemia was the statement made by some patients that the stools were unusually dark in color (not black). Hemoglobinuria was noted in certain of the fulminating cases (Chauffard and Vincent (40), Lederer (197, 198), Colarizi (56), Castex (39), Kaiser and Bradford (175), Nixon and Vaughn (248), and Landor (190)) and was particularly common in infancy.

Physical signs The patient's appearance when first seen is that of severe illness with prominent pallor. The temperature is almost always elevated and varies between a subfebrile type (99-100°F) to

high fever (103–105°F) The pulse rate varies between 100–140 per minute, the marked tachycardia being dependent upon the factors of anemia and fever The sclerae show definite icterus, and a yellowish tint of the skin is present Icterus is at times striking, but is usually only slight Most authors state that the pallor is more striking than the jaundice (“The patient is more pale than jaundiced”) The combination of marked pallor and moderate icterus give the patient a “lemon-yellow” appearance, similar to that seen in pernicious anemia Retinal hemorrhages may appear in the severe cases The tongue is entirely normal, possesses its normal coat, and shows no evidence of glossitis The lungs show no abnormalities The heart rate is rapid, and hemic murmurs, almost always systolic, are usually present, being located chiefly over the apical and lower precordial regions In Manne and Kuskin’s case (221) (a child), a diastolic murmur suggestive of aortic insufficiency was present, this disappeared as the patient improved The liver is frequently enlarged, although in Case 1 of our series it was felt to the level of the umbilicus The spleen, however, is almost constantly increased in size Of 40 representative cases, this organ was very readily palpable in 14, a firm edge being felt at least 2 finger-breadths below the left costal margin, and in occasional cases occupying almost the entire left upper quadrant In 14 cases, the spleen was just palpable In 12 cases, it could not be felt, although in 2 of these the organ was enlarged to percussion The spleen was readily felt in 3 of our cases, but could not be palpated in Case 3 The reason for this was obvious at operation which demonstrated that the organ, although twice normal in size, was adherent to the diaphragm At times, the spleen is definitely tender The remainder of the examination is usually negative, although slight edema of the ankles may be noted There is no lymphadenopathy The vibration sense is unimpaired and the knee jerks are normal

Laboratory data The *urine* is usually described by the patient as being dark in color Bilirubin, despite the icterus, is absent (acholuric icterus), so that the dark color is probably due to an excess of other pigments Urobilinogen and urobilin are usually increased in amount, the former pigment ordinarily being present in a dilution of 1:80–1:160 (technique of Wallace and Diamond (344)) Hemoglobinuria is at times noted, especially when the process is very acute, this finding may occasionally overshadow the remainder of the condition and

thus lead to the diagnosis of acute paroxysmal hemoglobinuria. In other cases, hemoglobinuria is not manifested as a gross disturbance but may be noted microscopically in the form of hemoglobinous casts. In still other cases, the symptom will only be found following a transfusion of blood, this may be due to sudden breakdown of donor's red cells by the patient's serum or vice versa. During the acute phase of the picture, albuminuria and casts are quite common.

The *stools* are highly colored. Discharges of bile are sometimes noted in the fulminating cases, due apparently to the combination of diarrhoea and excessive blood destruction. Urobilin is greatly increased in the feces, although but few quantitative determinations of this chemical constituent have been made in these cases.*

The *blood serum* is yellow in color and contains a greatly increased quantity of bilirubin. The icterus index is usually between 25 and 35 units, but higher values may be present. The bilirubin content of the blood serum is between 2 and 10 mgm per 100 cc. The bilirubin is of the "indirect" type, which correlates with the absence of bilirubin from the urine and the presence of increased urobilinogen.

The presence of hemolysins in the serum is the subject of comment below. Their discovery in Cases 2 and 3 of our series and the fact that hemolysins were described by Chauffard and Troisier (47), Chauffard and Vincent (50), Tixer (331), Roth (300), and others makes us suspect their presence in other similar cases. Demonstration of their presence is relatively simple and may be diagnostic in a given case.

The *blood cells*. The blood picture is analytically described below in the discussion of the blood picture. Giordano and Blum (121) discuss the highest and lowest red cell counts in the literature, the highest and lowest leukocyte counts, etc., and it is therefore unnecessary to repeat them here. Case 2 of Mendels (226) recent report deserves mention, however, in view of the erythrocyte count of 90,000 (!) which the author feels might have been due to autohemolysis occurring within the pipette. In our own cases, the first two showed a "pseudomacrocytic" type of anemia, i.e. (high color index, high mean corpuscular volume, but normal average red cell diameter) with

In two recent cases, the daily fecal urobilinogen output was 1313 and 621 mgs. respectively representing approximately 25 and 12 times the normal output (for the total mass of hemoglobin present).

erythrocyte counts in the neighborhood of 10 million (Figures 1, 11, 12) This type of blood picture which is said to resemble closely that of pernicious anemia has been reported by many investigators from the time of Widal (361 et seq) In our third and fourth cases (Figs 7, 34,

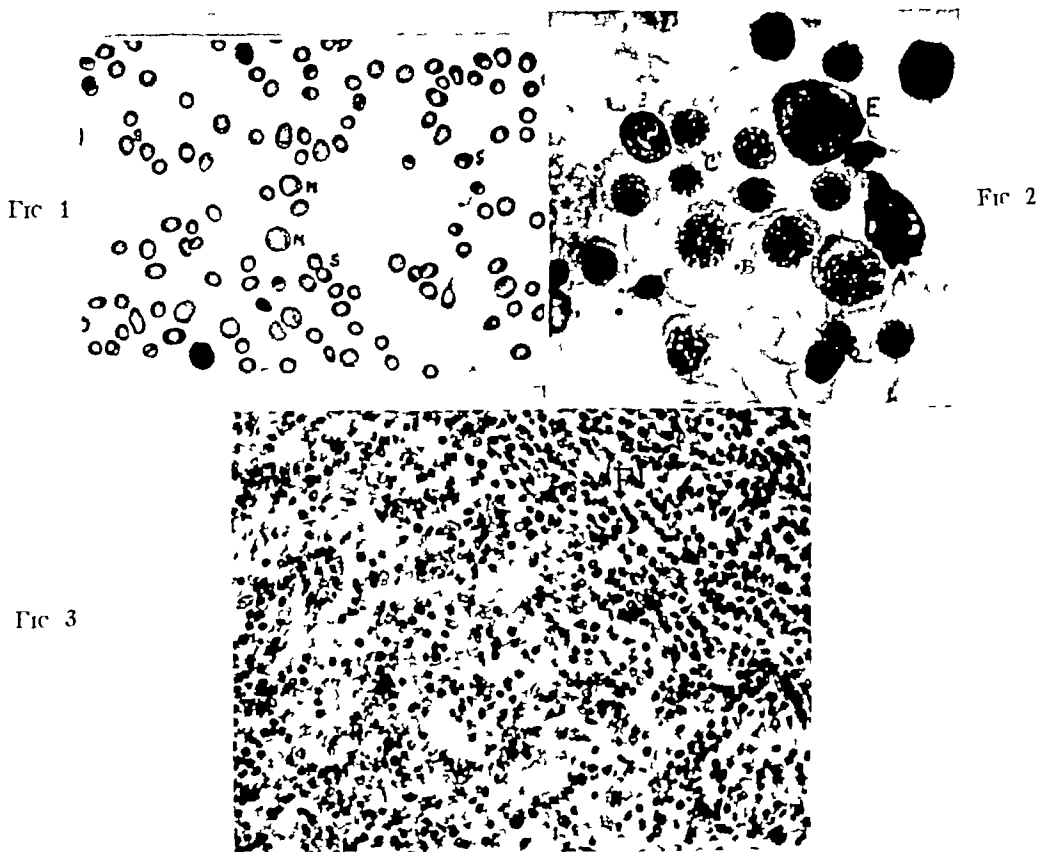


FIG 1 CASE 1 PHOTOMICROGRAPH OF BLOOD SMEAR ($\times 500$) ON ADMISSION MAY 20, 1937

Note the marked variation in size and shape of the red blood cells, the many small, dark-staining cells, i.e., spherocytes (*S*), and the frequent large cells, i.e., macrocytes (*M*). The finding of these large cells led at first to the erroneous diagnosis of pernicious anemia.

FIG 2 CASE 1 PHOTOMICROGRAPH ($\times 1000$) OF GIEMSA STAINED SMEAR OF STERNAL MARROW BIOPSY OF CASE 1

There was marked erythroblastic hyperplasia. A characteristic group of erythroblastic cells is seen. Note the erythrogone (primitive red cell—*E*) and the successive stages in maturation (normoblasts "*A*" and "*B*") to the mature normoblast "*C*" with pyknotic nucleus. The histologic picture of the red cells is normoblastic, differing from the megaloblastic hyperplasia of pernicious anemia.

FIG 3 CASE 1 PHOTOMICROGRAPH OF SECTION OF SPLEEN ($\times 360$)

Multiple small areas of thromboses and infarction are present. Note the fibrotic character of the splenic tissue contiguous to a follicle (*F*) with distortion of the splenic architecture.

22, 33, 13, 35, 23), the anemia was of the microcytic variety, although the mean corpuscular volume was normal (i.e. the thickness was greatly increased). In all four cases the reticulocytes were greatly elevated.



FIG 4

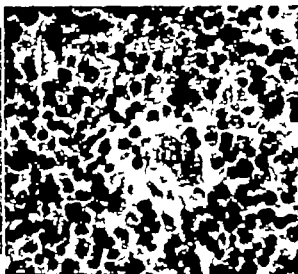


FIG 5

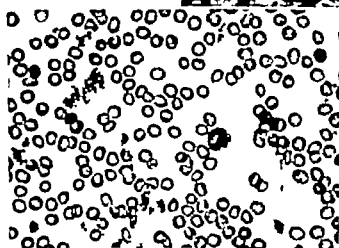


FIG 6

FIG 4 CASE 2 PHOTOMICROGRAPH OF SECTION OF SPLEEN ($\times 150$)

The follicles are relatively small and the remainder of the spleen is very cellular

FIG 5 CASE 2 PHOTOMICROGRAPH OF SECTION OF SPLEEN ($\times 800$)

The large pale staining cells are identified as reticulo-endothelial cells. Occasional giant cells are present and erythrophagocytosis is occasionally demonstrated

FIG 6 CASE 2 PHOTOMICROGRAPH OF BLOOD SMEAR ($\times 500$) 6 MONTHS AFTER SPLENECTOMY (FEBRUARY 1938)

Aside from slight achromia and slight anisocytosis, the blood picture is essentially normal

The leukocyte count was elevated in Cases 1, 3, and 4, and low in Case 2. Marked leukocytosis is characteristic of the very acute cases, the subacute cases, which have persisted for a month or longer, usually tend to develop increasingly low leukocyte counts, this was apparently the case in Case 2. The leukocytosis, which is polymorphonuclear in variety is, together with the reticulocytosis, a symptom of increased

bone-marrow regenerative activity Counts of approximately 50,000 are not unusual, particularly in children, and counts of 80,000 and

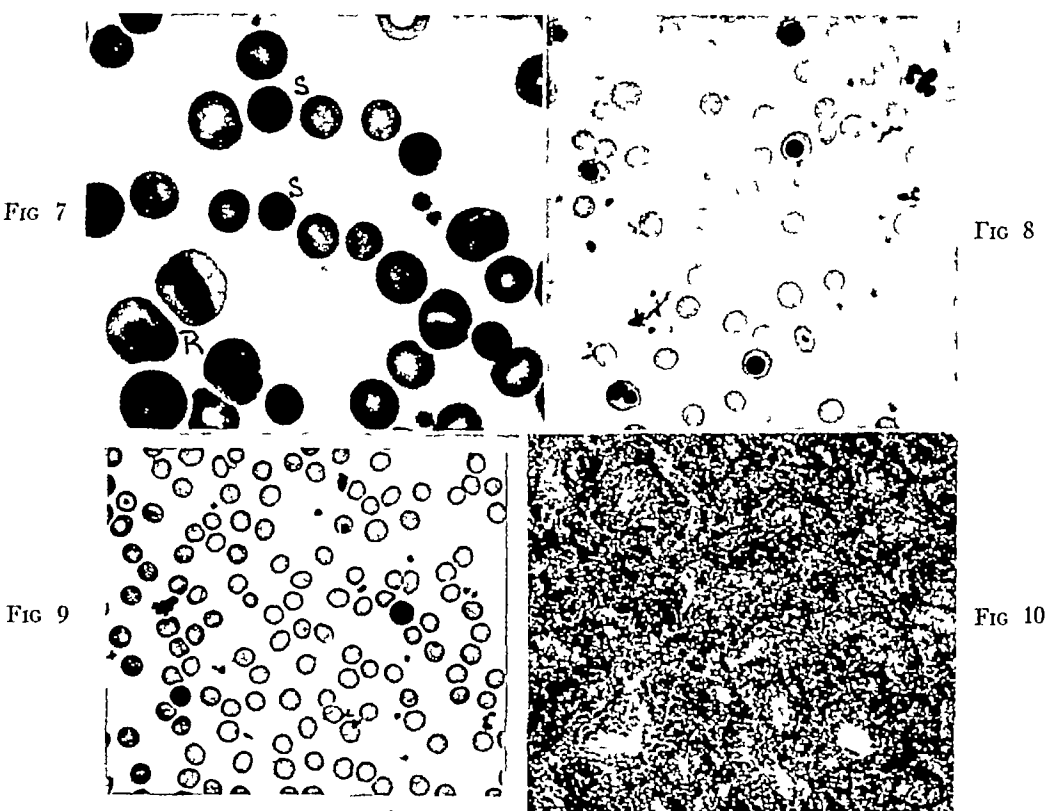


FIG 7 CASE 3 PHOTOMICROGRAPH OF BLOOD SMEAR ($\times 1350$) ON ADMISSION AUGUST 27, 1937

The contrast between the small, dense-appearing, dark-staining spherocytes (S) and the relatively huge reticulocytes (R) is readily seen

FIG 8 CASE 3 PHOTOMICROGRAPH OF BLOOD SMEAR ($\times 500$) SEPTEMBER 9, 1937, 11 DAYS AFTER SPLENECTOMY

After an initial rapid increase in erythrocytes, the erythrocyte production appeared to lag At this time, large numbers of nucleated red cells were present (maturation arrest?) Note the large numbers of platelets

FIG 9 CASE 3 PHOTOMICROGRAPH OF BLOOD SMEAR ($\times 500$) ABOUT A YEAR AFTER SPLENECTOMY (OCTOBER, 19, 1938)

Except for minor variation in size, the blood picture is essentially normal

FIG 10 CASE 3 PHOTOMICROGRAPH OF SECTION OF SPLEEN ($\times 140$)

There is an increase in the trabeculations and the sinusoids are widely distended Marked congestion of many areas with large numbers of erythrocytes was present.

over have been reported by Lederer (197, 198), Lazarus (196) and Teeter (324) Immature neutrophilic cells are almost constantly

present, and myelocytes are common, particularly in children. With myelocytes present, leukemia may be simulated (Cf Nobel and Steinebach (250)). Marked leukopenia is occasionally present (Manfredini (220), Goudsmidt (124), Plantevdt (273), Fiessinger (108) et al, and Chauffard and Vincent (40)).

The platelet count has only rarely been reported, probably because this hematological procedure is not usually performed. Heilbrun

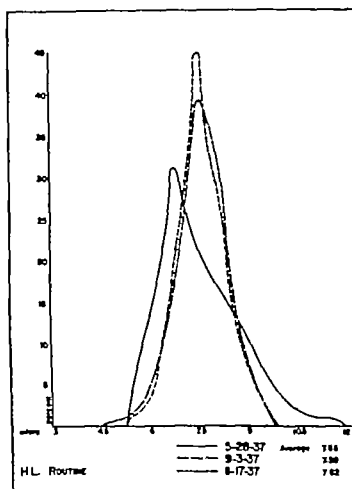


FIG 11 CASE 1 PRICE JONES CURVES OF RED BLOOD CELL DIAMETERS (NOTH RETICULOCYTES AND NON RETICULOCYTES)

Note the marked variation in size and the large number of microcytes present on 5/28/37 before splenectomy. The cells of 9-12 micra in diameter (macrocytes) were all reticulocytes and the blood picture was pseudo-macrocytic. After splenectomy the red cell population reverted to normal size.

found the platelets "reduced", Weil, Schreiber, and Cain (352) found them "numerous", Altmann (5) found 53,000 per cu mm, Christiansen (53) 352,000, Corcelli (58) 600,000, and Colarizi (56) found a "discrete number". In our own cases, the platelets were either normal or slightly diminished in number.

Pathology The spleen The condition of the spleen is described in 29 cases. The most detailed descriptions are given by Eppinger

bone-marrow regenerative activity
are not unusual, particularly

FIG 7

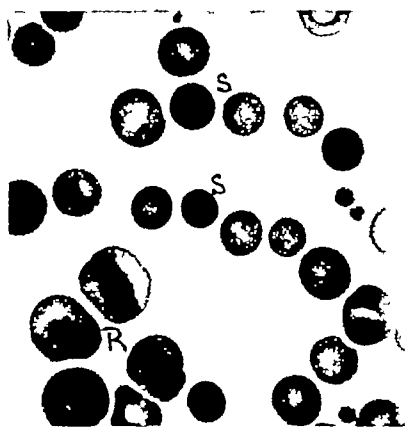


FIG 9

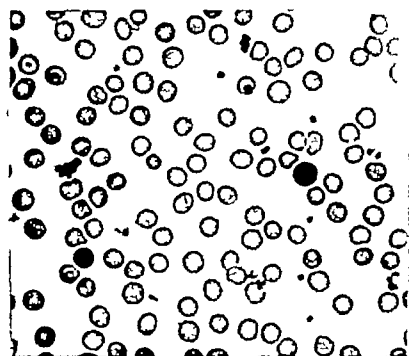


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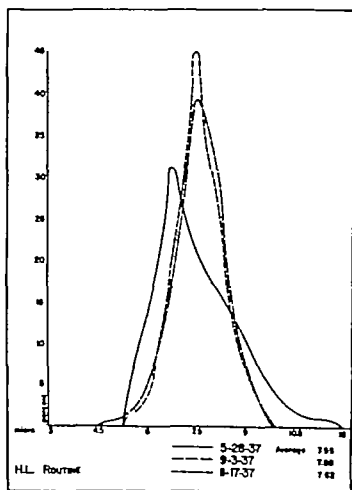


FIG 11 CASE 1 PRICE JONES CURVES OF RED BLOOD CELL DIAMETERS (BOTH RETICULOCYTES AND NON RETICULOCYTES)

Note the marked variation in size and the large number of microcytes present on 5/28/37 before splenectomy. The cells of 9-12 micra in diameter (macrocytes) were all reticulocytes and the blood picture was 'pseudo-macrocytic'. After splenectomy the red cell population reverted to normal size.

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(102) in his book on liver-spleen syndromes The organ was usually enlarged from $1\frac{1}{2}$ to 5 times its normal size In our own 4 cases, the splenic weights were respectively 550 gm, 500 gm, 360 gm, and 200 gm (the latter in a child aged 2 years) Histologically, they showed one of three separate types of abnormality similar to the cases reported

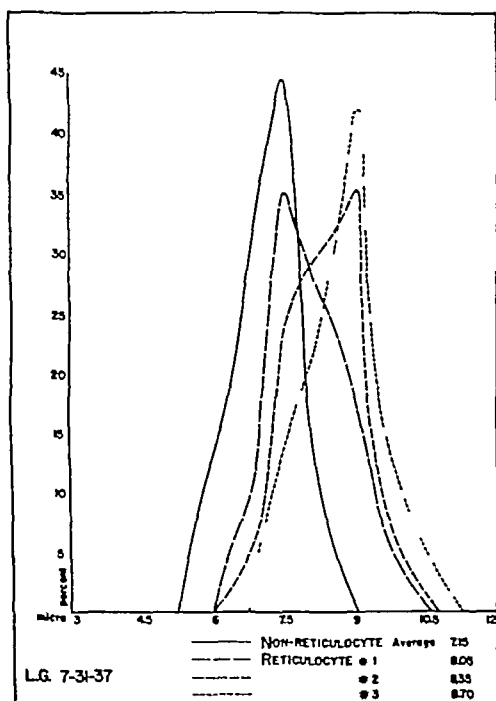


FIG 12 CASE 2 DIFFERENTIAL PRICE-JONES CURVES OF RETICULOCYTES AND NON-RETICULOCYTES

The reticulocytes are subdivided into 3 types #1—the most mature with only a few strands of reticulum, #3—the most immature with a fully complete network of reticulum in the middle of the cell, and #2, intermediate between the other types Note that the non-reticulated red cells tend towards microcytosis, and that the more immature the reticulocytes, the larger are the cells Since the most mature red cells tend to be much smaller than the cells closest to the marrow (immature reticulocytes), it is probable that specific smallness of cells (spherocytosis) develops outside the marrow

in the literature Thus Case 1 (Fig 3) was distinguished by numerous thromboses of veins and capillaries and with multiple venous infarctions of the pulp, Case 2 (Figs 4 and 5) by marked reticulum cell hyperplasia with giant cell formation and erythrophagocytosis, there was also an associated ectopic myeloid metaplasia Cases 3 (Fig 10) and 4 (Figs 16, 17) were characterized by extreme congestion

FIG 13

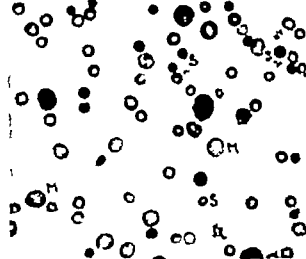


FIG 14

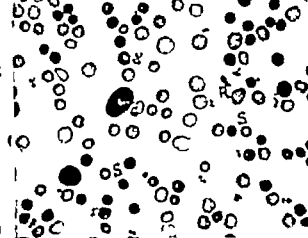


FIG 15

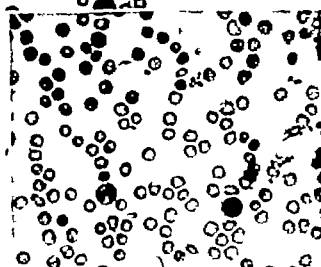


FIG 13 CASE 4 PHOTOMICROGRAPH OF BLOOD SMEAR (X500) MAY 27 1938
There is marked variation in size with many spherocytes (S) and not infrequent macrocytes (M)

FIG 14 CASE 4 PHOTOMICROGRAPH OF BLOOD SMEAR (X500) JUNE 7 1938
At this time hemolysis was outstanding and many spherocytes (S) were present. There was also marked regenerative activity as indicated by the marked reticulocytosis (R), thrombocytosis, and leukocytosis.

FIG 15 CASE 4 PHOTOMICROGRAPH OF BLOOD SMEAR (X500) SEPTEMBER 10 1938
ABOUT 2 MONTHS AFTER SPLENECTOMY

The red cell picture is much more normal although there is still tendency to spherocytosis.

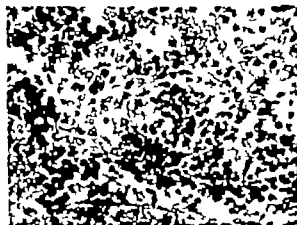


FIG 16

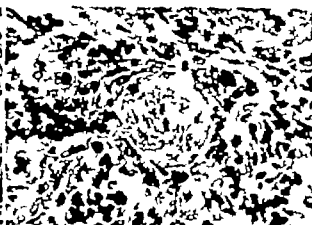


FIG 17

FIG 16 CASE 4 PHOTOMICROGRAPH OF SECTION OF SPLEEN (X500)
The splenic sinusoids are greatly distended and congestion and fibrosis of the remainder of the spleen is present.

FIG 17 CASE 4 PHOTOMICROGRAPH OF SECTION OF SPLEEN (X500)
An area of fibrosis is illustrated. In the centre is a fibrotic nodule.

of the pulp similar to that seen in congenital hemolytic icterus. Fibrosis and occasional fibrotic nodules were also a feature in Case 4 (Fig 17). In the cases in the literature, numerous infarctions of the spleen are reported by Lovibond (213), O'Donaghue and Witts (253) (2 cases), Shackle (311), and McGavack (216).

Histiocytic proliferation, at times with giant cell formation, and almost always associated with ectopic myeloid metaplasia was the outstanding feature in the cases of Antonelli (8), Anderson (6a), Davidson (68) (Case 6), Livingston and Edwards (211), Roth (300), Troisier and Catton (334), Rastetter and Murphy (282) (Case 1), and in the 4 cases of Israels and Wilkinson (167). Marked congestion of the sinusoids as the outstanding feature was present in the cases of Davidson (68) (7 and 9), Davidson and Fullerton (69) (Case 8), Goudsmid (124), Heilbrun (141), Payne (265), Rastetter and Murphy (282) (Case 2), and Rosenthal and Corten (296). Thus of 29 cases (including our 4) in which the splenic histology is described, histiocytic proliferation with giant cell formation and ectopic bone-marrow activity was prominent in 12, multiple infarcts in 6, and extreme congestion of the pulp in 11.

The bone-marrow The marrow has been described in 16 cases, at post-mortem in 9 and at biopsy in 7. The post-mortem observations are scanty and practically entirely limited to a gross description. Thus, a "currant-jelly" marrow was described by Shackle (311) and by O'Donaghue and Witts (253), "red" marrow by Goudsmid (124), a "maroon" marrow by O'Donaghue and Witts (253), (Case 2), a "grayish" marrow by MacIntosh and Cleland (218), and a "violaceous red marrow" by Widal and Weissenbach (369). Payne (265) describes a very hyperplastic marrow, Davidson (68) notes marked erythroblastic proliferation, and Widal and Weissenbach (369) describe a megaloblastic marrow with many myelocytes. Seven biopsies were performed by Corelli (58) (tibia)—few marrow cells were seen, by Rettani (287) (sternal puncture)—a normal picture with predominance of erythroblastic cells, by McGavack (216) (sternal puncture)—normal picture, by Tangredi (323)—hyperplasia, by Anderson (6a)—a "full" marrow, by Israels and Wilkinson (167) (Case 4)—marked hyperplasia, and by Heilbrun (141) (sternal trephine biopsy). In the latter case, the marrow was found to be very

cellular, with predominance of erythroblastic cells. Through the kindness of Dr Heilbrun, we were able to study his preparations, which showed normoblastic hyperplasia with many primitive nucleated red cells (erythrogonos). In our own cases bone marrow biopsies by the trephine method were performed in the first three. These showed in each instance a very hyperplastic marrow with marked predominance of erythroblastic cells (Fig 2). Many of the early nucleated red cells (Types "A" and "B") resembled megaloblasts, but careful examination demonstrated that the resemblance was only superficial. In any event, the most mature nucleated red cells (Type "C") were definitely normoblastic and showed none of the megaloblastic criteria. The marrow was thus "normoblastic" and could readily be differentiated from the typically megaloblastic marrow of the liver-extract deficiency state (pernicious anemia) (64). In Case 1 (H L), Price-Jones curves of the nucleated red cells of the marrow were made from stained smears. These showed the following average diameters for the various types of erythroblastic cells

	TOTAL DIAMETER	NUCLEAR DIAMETER
Erythrogonos	16.41	12.83
Normoblast A	13.58 (12.29)	10.26 (9.26)
Normoblast B	12.27 (11.09)	8.51 (7.08)
Normoblast C	10.64 (9.47)	5.88 (5.55)
Mature erythrocytes	7.50 (7.50)	

The figures in parentheses represent normal values and demonstrate that the normoblasts of this case were slightly larger than normal, although many of the mature red cells of the peripheral blood were spherocytes.

The leukocytes of the marrow showed no abnormalities, and misshapen metamyelocytes characteristic of the marrow of pernicious anemia were not seen.

Other organs. Of 10 post mortem examinations, enlargement of the liver with hemosiderosis was noted in almost every instance except that "acute yellow atrophy" was discovered in the case of Lovibond (213). A fatty heart was discovered in the cases of Shackleton (311) and Eimer (97). Hemoglobinous infarcts were found in the

case of Payne (265) (lethal transfusion reaction) The remaining organs showed little change from the normal

Diagnosis and differential diagnosis The diagnosis of acute, idiopathic, non-congenital, hemolytic anemia can either be simple or extraordinarily difficult The more or less sudden development of moderate to severe anemia, in association with slight icterus and moderate splenomegaly, should make one suspect the diagnosis Particularly is this true if there is no evidence of glossitis, combined system disease, an acute infection, hepatic disease, generalized lymphadenopathy, or recent exposure to chemicals or drugs Further evidence must be obtained from laboratory tests, particularly those of the blood and those indicating the presence of increased blood destruction The blood picture, as already outlined, usually shows the evidences of increased red blood cell destruction (anemia of the normochromic type, spherocytosis, increased fragility, abnormal rouleaux formation) together with the evidences of increased formation polychromatophilia, reticulocytosis, nucleated red blood cells, nuclear fragments, leukocytosis, polynucleosis, and thrombocytosis Careful supravital examination of the blood frequently shows the presence of small, thick, cup-shaped red cells, and of abnormal bizarre-shaped rouleaux (67a) Analysis of Price-Jones curves will show the presence of many microcytes as well as of macrocytes Differential reticulocyte-non-reticulocyte Price-Jones curves in our cases have shown that the microcytes are all mature, but that the macrocytes are for the most part reticulated The mean corpuscular volume is usually increased over normal, although in a fulminating case it may be diminished The mean corpuscular diameter is usually normal, although the *appearance* of macrocytosis may be given by the large number of polychromatophilic red cells True orthochromatic erythrocytes have only rarely been seen in our cases The mean corpuscular thickness is usually increased, sometimes greatly so

The blood serum is definitely more yellow than normal, (the bilirubin content being 4-6 times its normal value) and the urobilinogen content of the urine is greatly increased The bone-marrow at biopsy shows marked erythroblastic hyperplasia, the type of formation being definitely normoblastic

In the differential diagnosis, numerous conditions must be con-

sidered and ruled out by appropriate clinical or laboratory means. *Pernicious anemia* is usually first considered. In this disease, the tongue usually shows evidence of "inflammation," there is almost always definitely diminished vibration sense if not outspoken combined system disease, and the spleen is not usually palpable. In acute hemolytic anemia, the spleen is readily felt and there is no evidence either of glossitis or of neurological disease. Gastric analysis shows achlorhydria in pernicious anemia, and a normal or somewhat diminished hydrochloric acid content in acute hemolytic anemia. Although the blood picture has been stated by many authors to be identical in the two conditions, numerous differences are present. These are discussed under "The Blood Picture" below and are noted in Table 1. The sternal bone-marrow biopsy in pernicious anemia is characteristic and pathognomonic of that condition and related states (64). It is characterized by the presence of a peculiar megaloblastic type of red cell growth resulting in the formation of abnormally large mature erythrocytes. There is furthermore a very abnormal type of white cell growth with the presence of huge misshapen metamyelocytes. In acute hemolytic anemia, although primitive red cells are quite common, the final mature nucleated red cell is a typical normoblast with thick pyknotic masses of nuclear chromatin, abnormal metamyelocytes are not seen. A final differential point is of course the therapeutic test of liver extract which, although so remarkably effective in pernicious anemia, is without effect in hemolytic icterus. The therapeutic test, it should be stated, has usually been tried before the patient is seen in consultation.

Acute leukemia is often considered because of the rapid development of anemia, splenomegaly, and leukocytosis, often with the presence of many myelocytes. One of the chief features of acute leukemia is the presence of a hemorrhagic tendency which is due to gross reduction in platelets. These elements are either normal, increased, or slightly diminished in acute hemolytic anemia. Although myelocytes are common, most of the white cells are metamyelocytes and mature polymorphonuclears. This is quite in contrast with the "monotonous" type of blood picture seen in acute leukemia, in which practically all of the cells are primitive 'blasts with only an occasional myelocyte present.

TABLE 1

Differential Diagnosis of Acute Hemolytic Anemia and Pernicious Anemia

	ACUTE HEMOLYTIC ANEMIA	PERNICIOUS ANEMIA
History		
Onset	Subacute or acute	Insidious
Outstanding symptoms	Weakness, dyspnea, nausea and vomiting	Weakness, disturbances of the tongue, paresthesias, occasionally diarrhea
Physical examination		
Pallor	Marked, lemon-yellow	Marked, lemon-yellow
Icterus	Slight to moderate	Slight to moderate
Fever	May be high	Rarely high
Tongue	Normal	"Glossitis" almost always present
Liver	Enlarged in 25%	Rarely enlarged
Spleen	Usually readily palpable 3-4 fingers breadth below costal margin	Rarely felt
Neurological	Negative	Positive findings almost always present (at least disturbed vibration sensation)
Blood		
Fresh specimen	Abnormal "bizarre" rouleaux with all types of thick cells	Normal rouleaux
Anemia	Normochromic or microcytic	Macrocytic
Price-Jones curves	Marked variability with microcytes, the macrocytes are almost all polychromatophilic reticulocytes	Marked variability True orthochromatic macrocytes
Thickness studies	Spherocytosis, increased thickness usually present	No spherocytes No increase in thickness
Fragility test	Slight to marked increase in hypotonic sodium chloride fragility	Normal fragility
Nucleated red cells	Common, normoblasts	Rare, megaloblasts
White blood cells	Usually increased with many immature forms	Usually diminished, with multinucleated (overripe?) "P A" neutrophils
Platelets	Normal or increased	Moderate to marked diminution
Bone-marrow	<div> <div></div> <div> Marked red cell preponderance Maturation to normoblasts Normal metamyelocytes </div> </div>	Red cells and white cells increased Megaloblastic reaction Bizarre metamyelocytes
Gastric analysis	Free HCl present	Free HCl absent
Therapy	No response to liver extract	Response to liver extract

Lymphosarcoma, Hodgkin's disease, and even metastatic carcinoma may occasionally result in a hemolytic state indistinguishable from that of acute hemolytic anemia. Usually, the lymphoid masses of lymphosarcoma or of Hodgkin's disease are readily made out, but occasionally the disorder may be more central and limited chiefly to the spleen, bone-marrow, or mediastinum. The blood picture may be indistinguishable from that of the idiopathic type of acute hemolytic anemia. Such laboratory procedures as X-ray of the chest for mediastinal masses and X-rays of the bones for metastatic lesions might well be carried out while emergency treatment with transfusions is being given. If, because of the patient's lack of response to transfusion, splenectomy is considered, this is probably best postponed until a sternal bone-marrow biopsy has definitely demonstrated that the marrow is free of malignant cells.

The *hemolytic crisis of congenital hemolytic icterus* may be indistinguishable from acute hemolytic icterus. The family history is, however, usually well-known and the patient has usually known of the presence of the disease for some time. Microspherocytosis and greatly increased fragility are by no means pathognomonic of the congenital type, since they may occur (cf. our Cases 3 and 4) to as great an extent in the acute, non congenital disease. However, in the *absence* of a marked disturbance in fragility of the red cells one may exclude the hemolytic crisis of the congenital disease. We have thus far found free hemolysins in the serum only in acute hemolytic anemia and not in the cases of crisis of the congenital type. The absence of hemolysins does not, however, rule out acute hemolytic anemia of the acquired type. Hemolytic crisis may occasionally occur in Cooley's erythroblastic anemia and in sickle-cell anemia, the latter is readily distinguishable by the crescentic character of many of the cells, especially in fresh preparations. Cooley's anemia has usually been in evidence in a particular patient for some time, although its appearance in crisis is theoretically possible. If the diagnosis continues in doubt, X rays of the bones may show the peculiar osteoporotic appearance characteristic of the condition. *Acute hemoglobinuria* may occasionally be associated with acute hemolytic anemia, paroxysmal (cold) hemoglobinuria is distinguished by the presence of a positive Donath Landsteiner phenomenon and usually by a positive Wassermann reaction.

Sepsis, particularly of the streptococcic type, may result in fever, anemia, leukocytosis, splenomegaly, and the presence of icterus. The latter is usually of the "mixed" type indicating liver damage, the anemia is usually hypochromic, reticulocytosis is usually very slight, the polymorphonuclears show marked "toxic" changes, and there is neither spherocytosis nor increased fragility of the red cells. The blood culture is furthermore never positive in acute hemolytic anemia.³ The bone-marrow of sepsis is characterized by extreme white cell hyperplasia, most of the leukocytes being "toxic" in type. In contrast with the marrow of acute hemolytic anemia, the nucleated red cells are much reduced and primitive red cells (erythrogonos) are few (hypoplasia).

Treatment In the days (1907-1915) before transfusion and liver had been introduced as therapeutic procedures for the treatment of anemia, the treatment of these cases was usually unsatisfactory, although spontaneous cure at times resulted (Chauffard and Troisier (47), Chauffard and Vincent (50), Teeter (324), Widal, Abram and Brulé (364)). In other cases (Eimer (97), MacIntosh and Cleland (218), Tixier (331), Türk (337), Roth (300)) death ensued, although such remedies as Fowler's solution and iron were prescribed. Due chiefly to the dramatic effect of splenectomy, as noted by Micheli in "acquired hemolytic icterus" and by Banti (10) in "hemolytic splenomegaly," Antonelli (8), in 1913, recommended splenectomy in a case of subacute hemolytic icterus, this too resulted successfully. Nobel and Steinebach (250) reported the successful result of splenectomy in an acute case in 1914, and Eppinger (102) and Ranzi reported dramatic results in several cases. More recently, this procedure was apparently not attempted until 1931, when Lovibond (213), Davidson (68), and Troisier and Catton (334) reported cases. Transfusion of blood as a therapeutic measure for anemia, particularly of the "pernicious" variety, was utilized by many investigators beginning about 1915. The first to utilize it in acute hemolytic anemia were Widal and Weissenbach (369) who, apparently without the benefit of typing, gave their patient a direct transfusion from the artery of a

³ We have included in our series Case 8 of Davidson and Fullerton's recent study. In this case a *Salmonella* was recovered from the blood stream. Its relationship to the condition is questionable.

sister, death occurred. The use of transfusion in acute hemolytic anemia was first popularized by Lederer (197, 198) in 1925. Striking therapeutic results followed this procedure in three successive cases, this finding was confirmed in Lederer's second report also of 3 cases. Following Lederer's reports, most cases have been given transfusions, usually with excellent results. With the introduction of liver preparations in the treatment of pernicious anemia, these have been utilized in many of the cases, usually together with transfusions, sometimes alone.

Transfusions Transfusions of blood were given in 66 cases of those analyzed. The impression has gained ground that acute hemolytic anemia is a relatively benign affection which is dramatically cured by a single transfusion of blood. That this is often the case is undoubted, but multiple transfusions are frequently necessary in the severe or refractory case. In Brill's (30) case, 10 transfusions were necessary for recovery, in Lederer's (197, 198) 4th case, several transfusions were required before the patient made a very slow recovery, in Greppi and Semanza's (128) second case 5 transfusions were necessary, and in many instances 2 to 3 transfusions were required. A single transfusion of blood will often diminish the velocity of the patient's downhill course, but other transfusions will then be necessary to bring about continued improvement. Many authors have commented upon the striking success which often follows the infusion of even a small quantity of blood, thus in Altmann's (5) case (a child), 30 to 35 cc intramuscularly were followed by a dramatic change in the patient's condition, which was further ameliorated by repeated injections of this quantity of blood intramuscularly.

In some cases, transfusions have been valueless or of only temporary effect. This was the situation in our 4 cases, and has been noted by many others. In Payne's (265) case the patient died following transfusion, and in Parsons and Hawksley's (262) cases, hemoglobinuria and icterus became more pronounced after the first 2 transfusions. This also happened in case 3 of our series. Thus, although transfusions were followed by recovery in 44 of 66 cases, they were not curative in the remainder. Of these 22 cases, 4 died without further therapy, and in the remainder, splenectomy was done.

Splenectomy Splenectomy was first performed in a case of ac

quired hemolytic icterus by Micheli (231) in 1911 Banti reported successful therapeutic results with splenectomy in 2 cases of "hemolytic splenomegaly" Antonelli (8) in 1913 performed splenectomy in a subacute case of hemolytic icterus (anemia) with an excellent therapeutic result Nobel and Steinebach (250) reported the dramatic effect of splenectomy in 2 cases of "acquired hemolytic icterus" in 1914, one was acute In 1923, one of us (W D) observed the remarkable results of splenectomy in a case of acute hemolytic anemia following childbirth This patient had previously been given 7 transfusions of blood without effect, splenectomy was finally decided

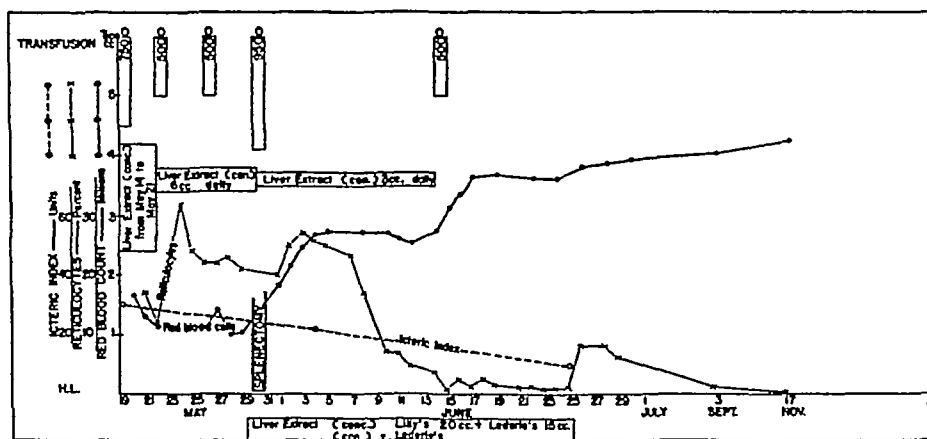


FIG 18 CASE 1 CHART OF HEMATOLOGICAL FINDINGS AND COURSE

Note the lack of erythrocyte response to liver extract and transfusions and the marked response following splenectomy There was a temporary flattening out of rapid rise approximately 1 week after operation Approximately 4 weeks after splenectomy, (June 25-29) there was a secondary rise in reticulocytes

upon as a measure of last resort and was followed by an exceedingly dramatic recovery When the first case of our present series was seen and the lack of response to transfusions and liver extract noted, splenectomy was proposed even though the patient was almost moribund Operation was followed by a rapid therapeutic response (Fig 18) Similar results took place in the three succeeding cases (Figs 19, 20, 21) and have also been described by Anderson (6 a), Antonelli (8), Decastello (71), Heilbrun (147), Israels and Wilkinson (167), McGavack (216), Livingston and Edwards (211), Rastetter and Murphy, Reynolds (288), Schwarz (309), Troisier and Catton (334), Davidson (68) (Case 7), and Davidson and Fullerton (69) (Case 8)

Lovibond's (213) case showed immediate improvement after splenectomy but unfortunately sepsis intervened and the patient died. This was also the situation in Cases 5 and 6 in Davidson's (68) series. Including our four cases, splenectomy has been performed in 23

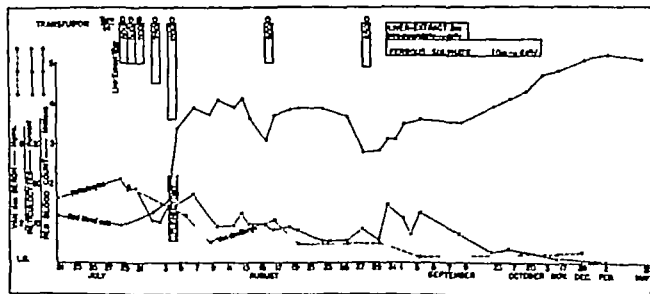


FIG 19 CASE 2 CHART OF HEMATOLOGICAL FINDINGS AND COURSE

Splenectomy was done when the inefficacy of repeated transfusions had been demonstrated. There was an immediate improvement clinically and hematologically. Further transfusions were given when reductions in red cell count appeared. Note the secondary reticulocytosis occurring approximately a month after splenectomy. Liver extract and iron were given because of the possibility that hemopoietic factors might be depleted. There was complete recovery.

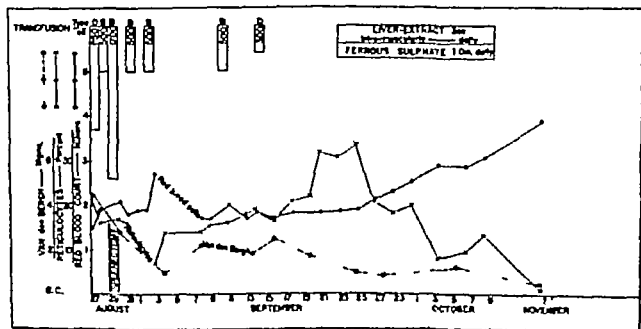


FIG 20 CASE 3 CHART OF HEMATOLOGICAL FINDINGS AND CLINICAL COURSE

This was a fulminating case and splenectomy was done as an emergency procedure after several transfusions. The immediate clinical and hematological improvement was not sustained so that further transfusions and the administration of hematopoietic substances seemed necessary. Note the secondary slight though definite increase in reticulocytes about 5 weeks after operation. There was complete recovery.

acute cases with recovery in 20 Death occurred from sepsis in 3

Liver extract Liver or liver extract has been given in most cases described since 1927 It has usually been administered together with blood transfusions so that its effect as a therapeutic procedure is difficult to evaluate In Case 1 of our series extremely large dosage of parenteral extract failed to cause any therapeutic response whatever, in Case 2, fairly adequate administration of liver extract caused no effect In Case 8 of Davidson's (68) series, the administration of liver extract and ventriculin seemed to cause slow improvement, which was then followed by an apparently spontaneous remission

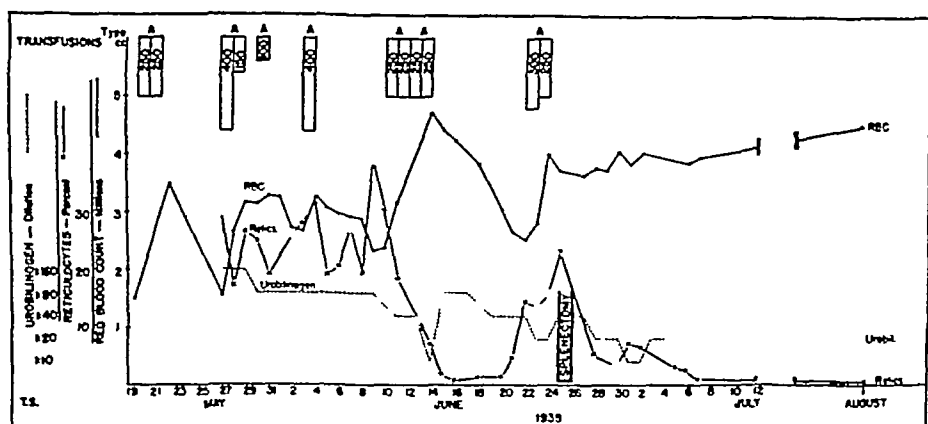


FIG 21 CASE 4 CHART OF HEMATOLOGICAL FINDINGS AND COURSE

Numerous transfusions resulted only in temporary improvement. After a final series of 4 daily transfusions, there was an extreme drop in reticulocytes (June 11-20) and only the hemolytic effect was visible (drop in erythrocyte count, increase in spherocytes, increasing red cell fragility, etc.) Splenectomy resulted in quick clinical and hematological response with complete recovery

In Benhamou's (17) case the patient was given 200 gm of calf liver for 40 days with recovery Christiansen (53), who gave his patient 300 gm of liver daily, stated that he considered the recovery to be spontaneous in origin and not related to the administration of liver Heilbrun (141) gave parenteral liver extract in his case but it is difficult to evaluate the response because a transfusion had previously been given Fiessinger (108) et al administered liver in their case for a period of almost a year, very slow improvement occurred In Lovibond's (213) case, reticulocytosis was present after the liver extract was given, the initial reticulocyte count, was, however, not known In Patterson's (263) case, that of a child, the administration

of liver extract was followed by a fairly prompt response. No response to liver extract, however, took place in the case of Troisier et al (334). Williams (371) gave his patient iron, copper, and liver extract, and recovery ensued. In summary, the results with liver therapy, as noted from the literature, are conflicting. The complete lack of response in several cases, the lack of clear-cut therapeutic response in any single case, and the probably coincidental or spontaneous rise in erythrocyte counts in several instances—all lead to the conclusion that liver preparations are of no value in the treatment of acute hemolytic anemia. Recapitulation of results obtained with the various methods of treatment is made below.*

		RECOVERED	DIED
Total number of cases	106	88	18
No or essentially no treatment (including liver extract)	24	13	11
Transfusions	66	44	4
Splenectomy*	23	20	3

* Most of these had been transfused without permanent effect.

III DISCUSSION

A Physiological pathology of the hemolytic syndromes

1 Normal physiology The cycle of the conversion of the iron-containing hemoglobin pigment into the iron-free pigment bilirubin has been abundantly studied by numerous investigators (13, 23, 102, 200, 289, 292, 301, 356). In brief, the effete red blood cells, after many thousand chemical exchanges during a period of about thirty days, become broken down and are then apparently phagocytosed by the cells of the reticuloendothelial system. Because the spleen contains the largest single compact collection of these cells, it is generally assumed that much of the red cell destruction takes place there, although the bone marrow, liver, lymph nodes, and other organs undoubtedly are very important in this regard. Hemosiderin is at first produced, and from it, by a series of steps as yet unknown, iron is

* At the time of the final correction of proofs (May 1940) 11 cases of acute hemolytic anemia had been observed. One case responded to transfusions. In 10 splenectomy was necessary. There was complete recovery in 4 cases, operative death in 4 cases (ages 50-64), and relapse in 2 cases. One of the relapsed cases died, the other made complete recovery after removal of a large dermoid cyst of the ovary.

liberated and the compound bilirubin formed. According to Barron and others, bilirubin in the blood stream, when first produced, is present in an adsorbed state, being bound to serum protein. This protein linkage is responsible for the "indirect" or "delayed" reaction with the Van den Bergh test, alcohol being necessary to "unlink" the bilirubin from its protein combination. The circulating adsorbed bilirubin is then taken up by the hepatic cells which concentrate the material for eventual passage into the bile canaliculi and then into the cystic and common ducts and gall bladder. Some change apparently takes place in the bilirubin as it passes through the liver cells—presumably by means of the bile acids—since in the bile the bilirubin is present in a "free" state and gives an "immediate" or "direct" reaction with the Van den Bergh test (13). Bilirubin is then excreted into the intestines where it becomes converted into urobilinogen and this in turn into stercobilin (urobilin). Some of this material is absorbed by the intestinal mucosa, appearing thence in the blood stream and being excreted by the kidneys into the urine. Bilirubin itself, although constantly present in the blood stream in appreciable quantity, is not normally excreted by the kidneys, apparently because the renal threshold does not permit the passage of adsorbed bilirubin (13). Urobilinogen is, however, readily passed by the kidneys and is found in the urine as such or in its oxidized form as urobilin.

Although the chief facts of blood destruction have thus been established, many remain unknown. Does the red cell in its final breakdown become fragmented, does it become hemolyzed, is it actually phagocytosed by the reticulo-endothelial system, if so, are certain enzymes or hemolysins involved in the process, does the spleen have any special function in the breakdown of the red blood cell?

2 Physiology of the hemolytic states As noted above, hemolysis is a normal event. There is constant breakdown of red blood cells, which is balanced by constant formation of new red blood cells, keeping the total red blood cell mass at a surprisingly constant level. Increased hemolysis from whatever cause results in an increased activity of the reticulo-endothelial cells and thus of the spleen. Presumably there is either hypertrophy of these cells or an increase in their number. The spleen becomes larger in volume because in addition to this reticulo-endothelial increase, its sinusoids become congested. An increased amount of circulating bilirubin develops, this is of the nor-

mal or "indirect" variety, since increased hemolysis is merely an exaggeration of the normal process. The increased amount of bilirubin which is produced is then secreted by the hepatic cells and thus the stools contain an increased quantity of stercobilin and the urine an increased quantity of urobilin(ogen).

An increased breakdown of red blood cells inevitably results in a stimulation of the bone marrow to compensate for this loss. Inactive marrow becomes active and normally active marrow becomes hyperactive. Erythroblastic hyperplasia within the marrow is indicated by a disproportion in the normal ratio between nucleated red cells and white cells, and by the presence of more primitive (erythrogones) and young forms of red cells (normoblasts "A" and "B") than are customarily seen. As is common in other conditions, with hyperplasia of the red cells series, the remainder of the marrow also becomes hyperplastic. These conditions within the marrow are reflected in the peripheral blood (see below).

Extremely rapid and violent hemolysis does not allow the normal processes of blood breakdown to take place in their characteristic progression. Under these circumstances, hemoglobin is found free in the plasma (hemoglobinemia) and becomes excreted in the urine as such (hemoglobinuria). This may be paroxysmal in type, in association with cold, or occurring chiefly at night, or after a long march or violent exercise. It may represent the only type of hemolytic process which is present in a given instance, although usually hemoglobinuria is associated with bilirubinemia. Thus paroxysmal nocturnal hemoglobinuria is accompanied by constant bilirubinemia and the various other phenomena of the hemolytic state. Hemosiderin, being an intermediary product between hemoglobin and bilirubin, may also be found in the urine in severe hemolytic states and has been described particularly in the paroxysmal nocturnal variety. Hemoglobinous casts are often found accompanying the hemoglobinuria.

Should the hemolysis be of a less severe and more chronic type, the regenerative process tends to keep pace with the process of destruction and the blood may present no striking deviations from the normal. The increased content of bilirubin in the plasma, of urobilin in the stools, and of urobilinogen in the urine, offer definite evidence of an increase in the breakdown of hemoglobin.

From the standpoint of the circulation, exceedingly rapid and ful

minating intravascular hemolysis results in a condition akin to hemorrhage or shock. The sudden reduction in erythrocyte count from 50 to 10 million is equivalent to the loss of approximately 3.5 liters of blood. This may give a clinical picture indistinguishable from that of acute hemorrhage or shock. The pulse rate becomes very feeble, the extremities may become cold and clammy, and semi-coma may develop.

It is thus possible to arrange in order a variety of hemolytic conditions, depending in all probability upon rapidity of hemolysis, beginning with the very mild congenital case on the one hand, and ending with the extremely fulminating state of violent paroxysmal hemoglobinuria (Chart 1) on the other.

3 *The pathogenesis of the hemolytic states with particular reference to hemolysins*. As mentioned above, the etiology of many hemolytic states is well known and may be due to such exogenous agents as bacteria (pneumococci, streptococci, *B. welchii*), protozoa (malarial parasites, trypanosomes), chemicals (lead, sulphanilamide, phenylhydrazine, toluidiamine), and more or less hypothetical toxins liberated by the fetus in certain cases of pregnancy. However, in the majority of the hemolytic states, both congenital and acquired, no exogenous hemolytic agents are demonstrable and because of this, much speculation has taken place regarding possible causative factors. In the early studies of acquired hemolytic icterus and before knowledge of the reticulo-endothelial system had been established, the liver was thought to be diseased. The occurrence of congenital and hereditary cases led many observers to suspect that an inherited or constitutional tendency was always present, but since this concept was frequently not explanatory, it was soon discarded. Because we have become impressed with the importance of hemolysins as aetiological factors in the hemolytic syndromes, we present below a somewhat extended discussion of this subject.

a *Immune hemolysins*. The finding by Chauffard (47, 50) and his collaborators of active hemolysins in the blood serum of 2 acute cases of the disease focussed attention on intrinsic hemolysins ("hemolysinemia") as possible factors. Donath and Landsteiner (80) had recently discovered a "cold" hemolysin in cases of paroxysmal hemoglobinemia. Coming in the wake of intense activity in the study of

hemolysins by immunologists, these observations created widespread interest. For many years it had been known that the transfusion of blood from one animal into another frequently caused a severe reac-

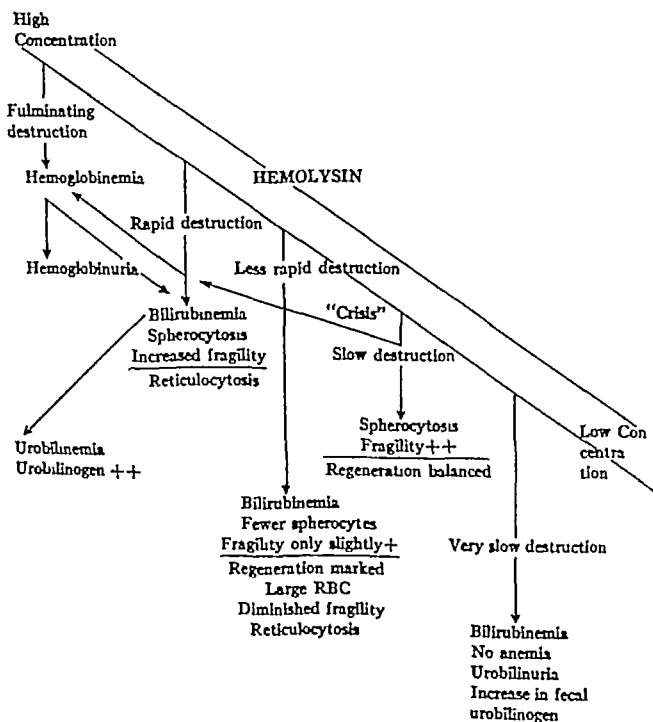


CHART 1 VARYING CONCENTRATIONS OF HEMOLYSIN AND CLINICAL HEMOLYTIC SYNDROMES

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minating intravascular hemolysis results in a condition akin to hemorrhage or shock. The sudden reduction in erythrocyte count from 5.0 to 1.0 million is equivalent to the loss of approximately 3.5 liters of blood. This may give a clinical picture indistinguishable from that of acute hemorrhage or shock. The pulse rate becomes very feeble, the extremities may become cold and clammy, and semi-coma may develop.

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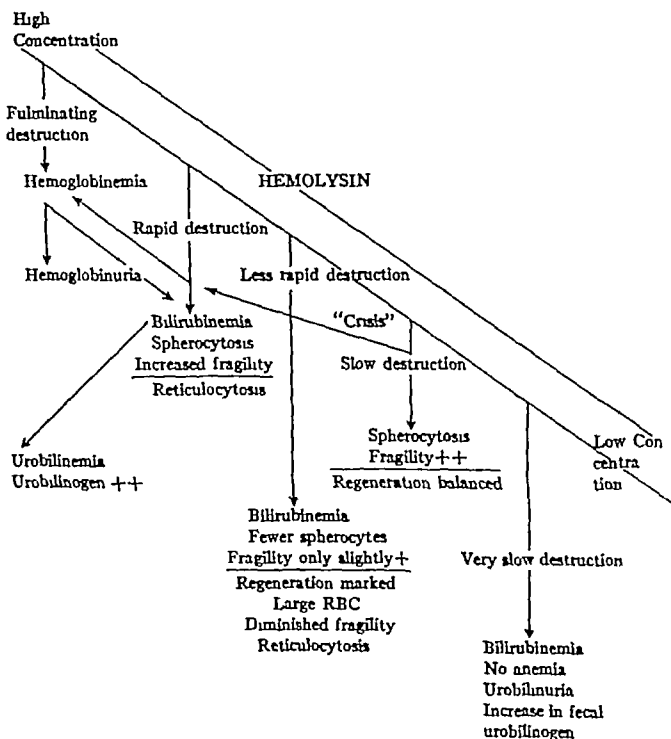


CHART 1 VARYING CONCENTRATIONS OF HEMOLYSIN AND CLINICAL HEMOLYTIC SYNDROMES

tion, which in the test tube manifested itself as lysis or laking. Buchner (34) concluded that the bacteriolytic and hemolytic actions of normal serum were due to the same constituent—"alexin" or comple

ment Bordet (27) showed that it was possible to produce an immune (hetero) hemolytic serum for rabbit red cells by injecting rabbit blood into guinea pigs. The hemolytic action of this serum could be destroyed by heating it to 56°C but "reactivated" by the addition of fresh normal serum (i.e. complement). This immune body was called "*substance sensibilisatrice*" by Bordet and "amboceptor" by Ehrlich (95). By means of a series of ingenious experiments, the latter investigator showed that amboceptor was able to attach itself in two ways—to complement in one, and to the red cell in the other.⁴

Iso-hemolysins in low titer have been demonstrated in human sera for many years. In animals, iso-hemolysins, like agglutinins, are not normally found. Attempts to produce them experimentally have usually been unsuccessful, although Ehrlich and Morgenroth (96), Lüdke (214), and Ottenberg and Thalhimer (259) produced them in goats, dogs, and cats respectively. The latter investigators noted the appearance of isolysins following several direct transfusions of blood from other cats. When blood from a normal cat was transfused to one which had developed serum isolysins, a severe hemolytic reaction occurred which was characterized by hemoglobinemia, hemoglobinuria, intravascular erythro-phagocytosis and leukocytosis. Lüdke (214) produced iso- and autolysins in dogs by first rendering them anemic by bleeding and then reinjecting the blood cells from the same dogs. In 9 of 11 experiments, iso- and autolysins appeared after an injection of blood. Most investigators have failed to confirm these findings.

In human sera, iso-hemolysins appear to be closely related to the normally present iso-agglutinins. Thus, Thomson and Thisted (328, 329) demonstrated that alpha and beta hemolysins were present normally in small concentration especially in Group O sera (i.e. sera containing alpha and beta agglutinins). The highest hemolysin titer they could discover was 1:10, although the agglutinin titer was often as high as 1:512. These workers also demonstrated that the alpha hemolysin was much more active than the beta type. Matsumaga (224) examined 315 human sera for the presence of iso-

⁴ For good general and detailed discussions of hemolysis and hemolysins, the reader is referred to the monograph of Sachs and Klopstock (305) and to Wells' "Chemical Pathology" (354).

hemolysins and made much the same observations. Determination of the presence of iso-hemolysins became difficult when the agglutinin titer was high and it was found impossible to separate hemolysin from agglutinin by differential precipitation. When the serum was heated to 50° for thirty minutes hemolytic activity disappeared, and reactivation could not be established by addition of complement. Matsumaga (224) also found that normal human serum contained an antilytic substance which became more active after heating the serum to 56° for thirty minutes and which could be completely destroyed by heating to 75°. No correlation was discovered between the presence of disease and the titer of hemolysin. "Isohemolysinogens" corresponding to the agglutinogens of the red cells have thus far not been studied.

b *The presence of hemolysins in hemolytic syndromes*. The first well-described hemolytic syndrome—paroxysmal hemoglobinuria—had early been shown by Donath and Landsteiner (80) to be associated with the presence of an hemolysin which became activated in the cold and then functioned at room temperature. In 2 cases of acute hemolytic icterus, Chauffard, with his collaborators Troisier (47) and Vincent (50) discovered active isohemolysins which functioned at room temperature. Various immunological tests demonstrated (1) that heating the serum to 56°C inactivated its lytic activity, (2) its activity could, however, be reestablished by the addition of guinea pig or horse-serum complement. (3) Iso-hemolytic activity of the serum gradually diminished as the patient improved, at first being present at room temperature, later requiring incubation for one-half hour. (4) The Ehrlich-Morgenroth phenomenon was positive. (5) The Donath-Landsteiner reaction was negative. At first red cells were chosen at random from 8 individuals and tested *in vitro*, the serum from the first case showed "brutal" (rapid and complete) hemolysis at room temperature in 7 instances. Later, hemolysis occurred in 9 of 11 experiments, but only at incubator temperature. Still later in the patient's course, tests for hemolysin were negative in 4 of 19 instances. Chauffard and Vincent (50) considered that the hemolysin probably possessed a definite pathogenic role. They insisted on its "precocious" character and on its "primary" origin within the body and believed that its presence was not merely secondary to red

cell destruction Unfortunately, Chauffard and his collaborators appeared to possess no knowledge of the blood groups, so that their observations are in this respect deficient Confirmatory observations were soon made by Troisier (332), Dufourt (85), Widai and Weissenbach (369), Guillain (130, 131, 132), Massagli (223), Tixier (331), and others v Stejskal (343) demonstrated the development of autohemolysis in cases of hemolytic icterus when the blood serum was kept in contact with the clot, control observations being negative This phenomenon, which took place in 8 to 12 hours at incubator temperature under sterile precautions, could be inhibited by heating the serum to 56°C for 2 hours v Stejskal also found that the complement content of the serum was low in hemolytic anemias Roth (300) in 1913 reported an acute case with death in which an autohemolysin as well as an autoagglutinin was demonstrated, although isolytic activity of the serum against the cells of other individuals could not be demonstrated A 10% suspension of the patient's red cells was hemolyzed in varying degree by 50 normal sera, but since agglutination also occurred with 48 of 50 of these sera, it is probable that the patient's cells were of Group AB Roth advanced the theory that hemolysins, although normally present in blood sera, did not become active unless abnormalities of the red cells were also present His conclusions, like those of Chauffard, were also considerably invalidated by lack of consideration of the factor of blood groups

Our own finding of hemolysins in 2 successive cases of acute hemolytic anemia led to careful study of the serum which showed the characteristics noted above As in Chauffard and Vincent's case the Ehrlich-Morgenroth phenomenon was positive and the Donath-Landsteiner reaction negative With recovery of the patient after splenectomy, the hemolytic activity of the serum gradually diminished

The concept that hemolysins were occasionally demonstrable in acute cases of hemolytic icterus was apparently completely forgotten in the period from about 1915 to 1938 In none of the cases of acute hemolytic anemia reported since 1925, and corresponding closely to the case of Chauffard and Vincent, was the presence of an hemolysin investigated, although in a few instances there was some speculation regarding its possible presence

In hemolytic syndromes other than the acute type of hemolytic anemia, hemolysins have occasionally been demonstrated The

Donath-Landsteiner phenomenon in cases of paroxysmal hemoglobinuria has already been mentioned. Salèn (306) discovered hemolysin in a case of transitory hemoglobinuria in which the Donath-Landsteiner phenomenon was negative, this hemolysin was not inactivated by heating to 59°C and complement was not required. Another type of hemolysin was described by Micheli (232), and by Macle, Wilkinson and Israëls (60) in cases of paroxysmal nocturnal hemoglobinuria. Ham and Dingle (141, 142) have recently made an exhaustive study of the immunological aspects of paroxysmal nocturnal hemoglobinuria. Although a definite abnormality of the red cell was discovered, the mechanism of the hemolysis was probably immunological in nature and required "the thermolabile components of complement derived from *human* serum". In these instances, the hemolysin was thermolabile and apparently non reactive to normal cells, although its presence was essential for the hemolysis of the possibly abnormal cells of the disease. Enneking (100) has described a somewhat different hemolysin in another case of hemoglobinuria. Lüdke found isolysins in 4 cases of hemolytic anemia—2 of the congenital and 2 of the acquired type. Autolysins were also found in two of these cases, but only during phases of hemolytic crisis when hemoglobinuria was prominent.

Tests for the presence of hemolysins in congenital hemolytic jaundice have only rarely been made, possibly because of the emphasis which has been placed upon the bone marrow abnormality in the disease. In the early studies of the French school, hemolysins were not found in the congenital disease. Both Beckmann (15) and Lüdke (214) in 1918 discovered hemolysins in congenital cases. Lüdke also demonstrated autolysins in his 2 cases. More recently, Green (125) found that normal washed erythrocytes became more fragile after incubation with the serum from a case of congenital hemolytic icterus. In 2 cases of the disease, we demonstrated serum isolysins which were active only against certain red blood cells. Thus in case H (Blood Group O) of 43 tests of the serum against various types of red blood cells, 35 were negative, 5 showed moderate hemolysis against Group O cells, 2 against Group A cells and 1 against Group AB cells. In Case C (Group O) of 15 tests, 7 were negative, 7 showed marked hemolysis (in 5 instances against Group O cells), and 1 was doubtful.

Thus, the occurrence of at least 4, possibly 5 types of hemolysins

has already been described in various types of hemolytic syndromes. It is probable, however, that not only are differences in hemolysin important, but that the effective dosage of hemolysin is of significance—as brought out in our animal experiments. In these, a large dose of hemolysin produced hemoglobinuria, a smaller one acute hemolytic icterus (anemia), and a still smaller dose a subacute type of anemia.

That free circulating hemolysins are not demonstrable in a given hemolytic syndrome does not necessarily rule out their presence within the body tissues. Wells (354), emphasizing a previous comment of Rous (301), points out that an injury insufficient to produce hemolysis under test-tube conditions may nevertheless bring it about in the animal body. An amount of serum hemolysin sufficient to lyse only a few cubic centimeters of blood *in vitro* may, when introduced into the organism, give rise to great destruction. This was well brought out in our animal experiments. Troisier's (332) experiments indicated that hemolytic amboceptor probably became directly fixed to the red cell. Guillaumin and Troisier (131, 132) stated that although two types of acquired hemolytic icterus could be discriminated (i.e. with and without the presence of hemolysins in the serum) these were not necessarily opposed. In the "hemolysinic" type the hemolysin was free in the serum, whereas more commonly, it was probably fixed to the cells. They agreed with Nolf (251, 252) that the first phase of hemolysis consisted in the production of hemolysin by body cells, the second phase in its fixation by the red blood cell. In the process of fixation, the cell might become damaged (Muir (210, 240), Wells (354)) (cf. section on Spherocytosis). Liebermann and Fenyvessy (210) believed that amboceptor first acted on the corpuscle, rendering it less resistant to subsequent complement—amboceptor activity.

c *Etiological relationship of hemolysins to the hemolytic process*
The finding of active hemolysins in various cases of acute hemolytic anemia focusses attention upon the possible etiological relationship of the hemolysin to the anemia. Chauffard and Vincent (50) concluded that a direct relationship was unquestionable. The possibility is present, however, that the hemolysin is produced when blood destruction is rapid, from whatever cause. Our own experiments (66, 67) have disproved this. Thus when distilled water or phenylhydrazine are injected intravenously and intraperitoneally in

rabbits there is no increase in the normal slight hemolytic titer of the serum despite the development of severe anemia. Diminution of the hemolytic titer in the sera of our cases simultaneously with improvement of the patients lends support to the hypothesis that the hemolysins bear some etiological relationship to the hemolytic state. Indirect evidence for this relationship is given by the activity of heterophilic hemolytic sera in the production of experimental hemolytic syndromes (66). Hemolytic sera were produced in rabbits by the injection of guinea-pig red cells. When these sera were injected intramuscularly or intraperitoneally into guinea pigs, a hemolytic process resulted, its severity depending solely upon the amount of serum injected (Fig 29). A large dose of serum of high titer resulted in hemoglobinemia, hemoglobinuria, and quick death, a moderate dose in the rapid development of acute hemolytic icterus with severe anemia, spherocytosis, increased fragility of the red cells, and evidences of very marked red cell formation (reticulocytosis, nucleated red cells). Subacute pictures in which a "pseudo macrocytic" type of blood picture was present (similar to that seen in our first two cases) could be produced by smaller doses of hemolytic serum. It should be noted that even with the injection of large doses (0.5 cc) of hemolytic serum of high titer with resultant severe anemia, a hemolysin could not be detected when the blood serum of the guinea pig was tested for hemolytic activity. These observations serve to indicate (1) that a serum containing an immune hemolysin can produce various types of hemolytic syndromes similar to those seen in man, (2) that (conversely), since an hemolysin similar in immunological characteristics is found in certain human cases of acute hemolytic anemia, it may be of etiological relationship to the disease process.

From the physiological standpoint, the presence of hemolysin in our cases may be explained on two quite different grounds: (1) that it represents an "overflow" mechanism, (2) that the hemolysin is indeed "precocious" and of a different chemical nature than the alpha and beta hemolysins of immune type. In favor of the "overflow" mechanism is that with the hemolytic process extremely active, *in vitro* hemolysis was "brusk" ("*brutale*" to use Chauffard and Vincent's expression). This might have been due to so great an excess of hemolysin that even Group O cells were hemolyzed. As far

as we have been able to ascertain, in no other instances than ours have isohemolysins been demonstrated which are hemolytic for cells of Group O. Theoretically, this finding is impossible since Group O cells, being without agglutinogens, are in all likelihood without "hemolysinogens." Whether or not this feature serves to set our hemolysin apart from the immune types of hemolysins which have previously been described is debatable. It should be noted, however, that as the extreme "bruskeness" of the reaction lessened, hemolysis took place only with A and B cells, indicating possibly that alpha and beta hemolysins were the original types present. That the hemolysin was of an entirely different order from those ordinarily seen is not likely since not only is this finding unique, but it postulates either a purely chemical type of reaction or else an hemolysin of organic nature which does not require an "hemolysinogen" for its activity. A chemical type of reaction might be conceivable, but since the hemolysin otherwise behaved in orthodox immunological style, this is unlikely. It is our conception, therefore, that the hemolysin, despite its unique features, was probably not "precocious" but of such high titer that it was capable of hemolyzing red cells devoid of hemolysinogen.

d *Autolysins* The criticism may be made that although isohemolysins in the serum of one individual hemolyze the red blood cells of another, and that heterohemolytic sera can produce a hemolytic process in animals, their etiological relationship to a clinical hemolytic process is not necessarily demonstrated. Of greater importance would naturally be the finding of an hemolysin active against the patient's own cells, i.e. an autohemolysin.

Although autolysins have occasionally been reported, systematic examinations for their presence were unfortunately not performed in our cases. In Case 3, however, marked autolysis of the blood clot occurred at ice box temperature when the blood was kept overnight. Chauffard and Vincent (50) reported slight autolysis in their case. Reference has already been made to the findings of v Stejskal (343), Roth (300), and Lüdke (214). Despite the rather consistent lack of demonstration of serum autolysins in various hemolytic processes, their presence within the body tissues is still a possibility, and, as stated above, the abnormalities (particularly spherocytosis) of the red blood cells might be due to their previous activity. Although the finding of isohemolysins in various hemolytic processes does not carry

with it the significance entailed in the finding of an autolysin, it must nevertheless be admitted that their demonstration is etiologically suggestive and in all probability not coincidental

e *Autoagglutinins* In Cases 2 and 3 of our series the agglutinin titer of the serum was highest when the patient was seriously ill, and became lower as the patient improved. Unfortunately, tests for autoagglutination were not made*. Widal and his collaborators, in their reports on acquired hemolytic jaundice pointed out that autoagglutination of the red cells was a constant phenomenon, both in the acute and chronic relapsing cases. They stressed the diagnostic importance of this phenomenon and pointed out that this finding, together with the slight disturbance in fragility, offered two objective criteria helping to differentiate the acquired from the congenital type of hemolytic icterus. The observations of Widal *et al* were soon confirmed in many reports on acquired hemolytic icterus but were again (as with the isohemolysins) apparently forgotten. Thus, Giordano and Blum (121) recently stated (1937) that theirs was the first finding of autoagglutination in a case of acute hemolytic anemia and this statement was also accepted by Greenwald (126). Of the cases included above in our analysis of the literature, autoagglutination was searched for and found present by Antonelli (8), Roth (300), Troisier and Catton (334), Davidson (68) (Case 2), Patterson and Smith (264), Giordano and Blum (121), and Greenwald (126) (Case 2). The common finding of autoagglutination in the cases of acquired hemolytic icterus (all types) reported years ago is another link in the establishment of the concept that acquired hemolytic icterus (acute type) and acute hemolytic anemia are one and the same disease.

Many immunologists have commented upon the close relationship which exists between the isoagglutinins and the isohemolysins, as well as upon the fact that when the agglutinin titer is greatly increased, titration of hemolysins becomes exceedingly difficult. Thus, in testing for hemolysins, if the serum has marked agglutinating properties, hemolysis of the agglutinated red cells will be prevented although the hemolysin titre is high. This fact has been abundantly recognized and attempts, thus far unsuccessful, have been made to

* In a recent case (L.), a "pan" agglutinin with autoagglutinating properties was present in high titre, making blood-grouping and cross-matching almost impossible. The agglutinin was most active at ice-box temperature, and gradually diminished as the patient improved.

absorb out the agglutinins from a serum before testing it for hemolytic activity. Several workers (332, 85, 32) have pointed out that the high agglutinin and autoagglutinin titre of cases of acquired hemolytic icterus might be indirect evidence of the presence of high hemolysin and autohemolysin titres in the serum. It is thus not unlikely that increased agglutinin titres and the presence of autoagglutinins might indicate the presence of a hemolytic process. For example, autoagglutination has also been reported in paroxysmal (cold) hemoglobinuria (Kopplin (179), Mino (235)), and autoagglutinins acting only at low temperatures by Patterson and Smith (264), and by Rosenthal and Corten (296), in cases of acute hemolytic anemia. As with the isohemolysins, the cause for a marked increase in agglutinin content of the serum is not apparent. It should be remembered, however, that the exact nature and site (or sites) of the production of the ordinary type—specific isoagglutinins have thus far not been elucidated.

f *Other possible factors* (complement activity, diminished antilytic activity). Various observers have commented upon the possibility that changes in complement value of the serum may have some bearing upon its hemolytic potency (89), and that this feature might be of importance in hemolytic anemia. The titre of complement has been stated to be low in congenital hemolytic icterus (330). Others have considered that the serum of hemolytic anemia might be deficient in normal antilytic activity. This possibility was investigated by Clark and Evans (54) in 1920, and by Zinck, Clark, and Evans (379) in 1922 (recently by Murphy and Howard (242)), who found that the serum of patients with hemolytic anemia and pernicious anemia was deficient in its normal protective power against the hemolytic activity of saponin and sodium oleate. Our own experiments demonstrated the pronounced antilytic activity of normal human serum against the lytic activity of the sera from Cases 2 and 3 of acute hemolytic anemia. Kraus and Clairmont (181) and Besredka (21) demonstrated many years ago that normal human serum was antilytic and the latter postulated a normal balance between lytic and antilytic processes. This work was confirmed by Neisser (246), Muller (241), and Camus and Pagniez (38). For their antilytic activity Josephs (172, 173) has recently utilized concentrates of normal swine blood serum in

the treatment of various hemolytic states. The results so far are inconclusive. Attempts to produce an active antilysin (an "anti-antibody") have thus far been unsuccessful, although Ehrlich and Morgenroth (96) claimed to have done this in goats. Preliminary experiments of our own in rabbits have been unsuccessful. The possibility of a peculiar type of allergic reaction should be considered, especially in view of the fact that one type of acute hemolytic process (favism) has been shown to be due to a hypersensitivity to the fava bean.

g The possible site of origin of hemolysins Dating from Minkowski's report, the spleen has assumed the central point in speculations regarding a possible site of a hemolytic agent. Metschnikoff (227), Korschun and Morgenroth (180) (1902), and later Gilbert, Chabrol, and Benard (119, 120) and Nolf (251, 252), found that splenic extracts possessed hemolytic activity due not to an amboceptor-complement reaction but to hemolytic substances presumably of cellular origin. Banti (10) attempted to investigate the hemolytic function of the spleen by the experimental production of hemolytic anemia with heterophilic sera. He found that the fragility of the red blood cells was greater in blood from the splenic vein than that from the general circulation. Hemoglobinemia, when present, was also greater in the splenic vein. These observations were confirmed by several workers. The hemolytic process which occurred when hemolytic sera were used was slighter in splenectomized animals and took longer to develop. Extracts prepared from the spleen of normal dogs possessed either slight or no hemolytic activity, on the other hand, splenic extracts from dogs with experimental hemolytic anemia possessed a definite hemolytic activity. Histologic studies of the spleen in the experimental animals furthermore demonstrated marked congestion of the pulp, erythrophagocytosis, increased size and function of the reticulum cells and various gradations in the breakdown of the red blood cells. Finally, the striking result of splenectomy in his case of "hemolytic splenomegaly" added great weight to the experimental and speculative observations, and Banti concluded that the spleen was the principal organ of hemolysis, probably through the medium of hypothetical cytohemolysins produced by the splenic cells. He also felt that the many resemblances between human and experimentally produced hemolytic anemia were too striking to be ignored. The origin of the

hemolytic agent which caused the initial stimulation of the spleen and its resultant hemolytic activity was a factor which could only be speculated upon

Antonelli (8) (1913) criticized many of Banti's views in discussing a case of his own. On the basis of clinical experience, a thorough review of the literature, and a few experimental observations, Antonelli concluded that although splenopathy was undoubtedly a pathogenic factor of great importance in the disease, it could not be implicated as the sole or even the most important factor since the hemolytic agents might also be present in other viscera. Furthermore, splenectomy, although dramatic in its effects, might cause them simply by reducing red cell destruction rather than by removing the organ producing the hemolysin.

v Stejskal (343) (1909) found that autohemolysis could be demonstrated in certain cases of hemolytic anemia by allowing the serum to stand in contact with its clot at incubator temperature for several hours. In cases of hemolytic jaundice this worker produced stasis of the arm by means of a tourniquet, and found that blood removed from a congested area showed increased autolytic activity, whereas blood removed from an anemic area, rendered so by keeping the arm up, showed diminished lytic activity. v Stejskal thus reasoned that hemolysis was favored by congestion and since stasis might readily occur in the spleen, hemolysis might take place there. He posed the question at this time whether hemolysis could be explained upon a purely physical basis such as stasis or upon the basis of the activity of hemolysins. Since his experiments pointed to the importance of both these factors, he concluded that the important feature of a given hemolytic process was *either* a physical or chemical process acting upon the red blood cells and lessening its resistance. Bergenhem and Fahreus (18, 19) recently presented somewhat similar views. These investigators felt that the phenomenon of spherocytosis might be due to the presence of a "lysolecithin" in the blood which was chiefly active in areas of sluggish circulation where an "endopause" could occur. Because of the well known storage of blood in the spleen and its sluggish circulation, they felt that the spleen was in all probability the chief "endopausal" organ. Singer (314) has confirmed many of the experiments of Bergenhem and Fahreus and comes to

the same general conclusions regarding the importance of the spleen in "lysolecithin" production

These various speculations are attractive and may be said to build up a formidable case for the relationship of the spleen to hemolysin production. The undeniably rapid and dramatic response to splenectomy which occurs in many cases immediately implicates the spleen. This is a "mysterious organ," however, and the effect of splenectomy need not necessarily mean the removal of an hemolysin-producing organ but rather one which acts in a more mechanical manner. The problem is by no means simplified by study of the histology of the spleen in these cases. As noted above, three types of picture were discriminated: (1) congestion (2) infarction and (3) histiocytic proliferation. Simple physiologic hyperactivity in the breakdown of red blood cells might account for any or all of these pictures. From study of the histology alone in these cases it is impossible even to speculate regarding the splenic physiology.

Similar problems are presented in thrombocytopenic purpura—does splenectomy cause sudden dramatic response because of the removal of a thrombostatic organ or because the organ inhibiting megakaryocyte-platelet formation in the bone-marrow is removed? The effect of splenectomy may simply be due to the removal of the largest single aggregation of blood-destroying cells.

The hemolytic factor, whether cellular or serological in type, may be said to cause changes in the red cells (spherocytosis, increased fragility) rendering them more readily destructible by the normal organs of blood destruction. Heilmeyer has recently stated such views (148, 149). In the presence of an increased number of cells to destroy, the spleen may become increasingly large, the large size may thus be an index merely of increased physiological, rather than pathological, activity. Splenectomy will thus diminish hemolytic activity in any event and may allow weakened red blood cells to remain in the circulation for a longer time without being destroyed. Banti's experimental observations are in line with this supposition. In our own cases, hemolysin was demonstrable to an appreciable extent in the serum long after splenectomy had been performed, this tended to show that hemolysin was not produced entirely by the spleen. Perhaps the entire reticulo-endothelial system was implicated.

The *immediate* effect of splenectomy is however so striking a phenomenon that it cannot be dismissed by stating that the largest blood-destroying organ is removed. As Curtis, Doan, and Wiseman (59) have pointed out in their discussion of emergency splenectomies for hemoclastic crises, the effect is indeed immediate, occurring even on the operating table. This makes one postulate a more *positive* function of the spleen than the simple destruction of weakened red blood cells in these cases. Lauda distinguishes between an "active" and a "passive" hemolytic function on the part of the spleen (193, 194, 195)⁵. The fact that splenic extracts are more hemolytic than extracts of other organs does not help to elucidate the problem. We may conclude that the site of origin of a hemolytic factor is *sub judice*—that it may well reside in the spleen—that the spleen probably has a "routine" or "passive" hemolytic function as well as a possible "active" one—that in any event, splenectomy is often successful in inducing an immediate cessation of hemolysis.

h Possible initiators of hemolytic activity The fundamental origin of the stimulus for hemolysin production must as yet remain unanswered. A hetero-hemolysin may be produced experimentally by sensitizing one animal to another animal's red cells. As noted above, a few investigators have demonstrated that an isolysin may be produced by injecting animals with large quantities of blood from the same animal species. It is theoretically possible that the body may on occasion become sensitized to its own red blood cells. This might be in the nature of an allergic or immune process as the result of a previous infection or in response to the introduction of an anti-red cell substance. The possibility of sensitization to the various breakdown products of the red blood cell is present. Another possibility again centres about the spleen. Rapid changes in the size of this organ are known to occur. With sudden contraction, a fairly large amount of blood may be suddenly introduced into the circulation. That events of this type might conceivably result in the building up by the body of autolysins is not altogether impossible. The opposite of this reaction—i.e. chronic distension of the spleen with stagnation of blood and the resultant building up of appreciable quantities of lysins

⁵ For an excellent discussion with complete bibliography of the spleen as an hemolytic organ, the reader is referred to Lauda's monograph (194) on the physiology of the spleen.

is another possibility. Chronic splenic stasis might also result in a more mechanical type of increased hemolytic activity. Troisier (332) in his thesis on hemolysins showed that if hemorrhage occurred into a body space (hemothorax, hemoperitoneum) hemolysins appeared in the blood and gradually increased in titer. The hemolysin then became fixed to the red cells rendering them more fragile.

It is possible that certain infectious agents might directly result in the development of hemolysins, especially in view of the fact that various types of hemolytic organisms as well as bacterial lysins are well known. The theory advanced by Lederer (197, 198) that since fever and leukocytosis were present, an infectious process was probably causal, must be discounted since active blood destruction and marked anemia from whatever cause are both associated with fever. This is particularly true in severe hemolytic crises such as occur occasionally in pernicious anemia, following transfusions of blood in congenital hemolytic icterus, and in the hemolytic accidents of phenylhydrazine and sulphanilamide poisoning. That infections may, however, initiate the development of increased hemolytic activity on the part of cells which are normally productive of hemolysins cannot be denied.

*B The blood picture**

The spherocytosis Fundamentally, the blood picture of acute hemolytic anemia is a mixture of two opposing sets of phenomena: those (1) of increased blood destruction and (2) of increased blood formation. The degree of blood destruction, and its velocity, can frequently be measured not only by the rate of fall in hemoglobin and red cell count but by the degree of spherocytosis present. The concept that the spherocyte is an indicator of intravascular hemolysis is a new one and deserves some comment. Most observers have pointed out the marked anisocytosis and the large number of microcytes which are commonly present. Our studies have demonstrated that the microcytes of acute hemolytic anemia, like those of congenital hemolytic icterus, are for the most part thicker and rounder than normal, i.e. spherocytes. Spherocytosis in our own cases was most marked in

* For illustrations of the various comments regarding the blood picture the reader is referred to the photomicrographs and Price-Jones curves of our cases (cf. Figures 1, 11, 12, 6, 7, 34, 8, 9, 22, 33, 13, 35, 23, 24, 25, 14, 15, 27).

Case 3 with the fulminating course—and least marked in Cases 1 and 2 which were subacute in type and of at least a month, probably longer, in duration In Case 4, which we were able to observe closely during several successive relapses, the degree of spherocytosis went hand in hand with the other indices of increased hemolysis, i.e. anemia, increased bilirubinemia, and increased output of urobilinogen

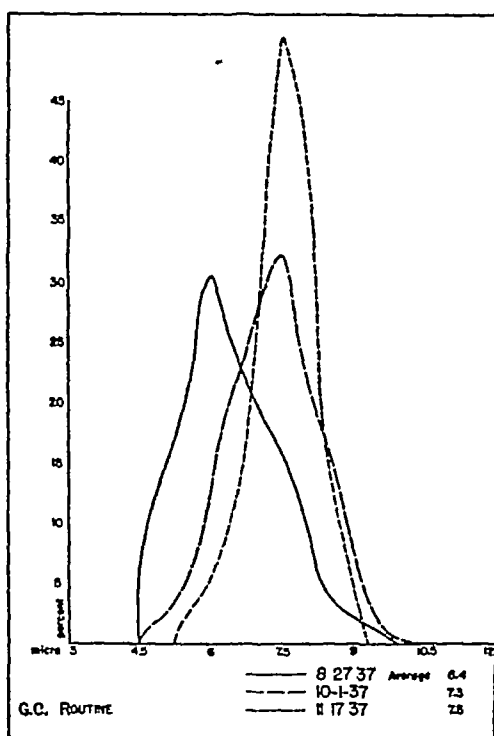


FIG 22 CASE 3 PRICE-JONES CURVES OF RED CELL DIAMETERS (INCLUDING RETICULOCYTES AND NON-RETICULOCYTES) BEFORE (8/27/37) AND AFTER SPLENECTOMY

Note the extreme degree of microcytosis on the first date, the larger cells are all reticulocytes (see figure 18) On 10/1/37 there was still a tendency for microcytosis but on 11/17/37 the curve was essentially normal

The spherocyte is a red blood cell which is smaller and thicker than normal In stained blood smears it appears to be excessively dense and of a deeper red color than the remainder of the red cells It is never polychromatophilic and spherocytic reticulocytes are rarely seen The spherocyte is best studied in the fresh blood smear made by dropping a cover slip containing a small drop of blood upon a clean glass slide Excellent preparations have been obtained with the use of our platelet-reticulocyte solution (Dameshek (63)) With this method,

various gradations in thickness of the cells are seen (Fig 17) The first change from the normal is the loss of one of the normal biconcavities and the formation of a cup shaped red cell This then be-

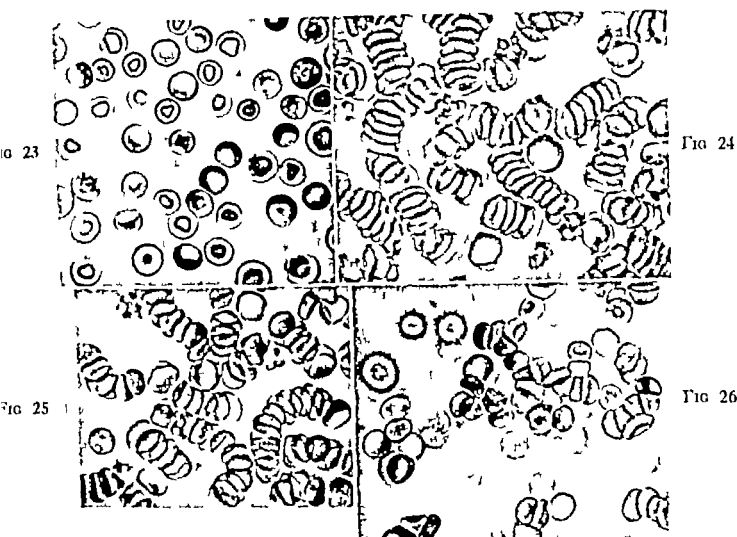


FIG 23 CASE 4 PHOTOMICROGRAPH ($\times 1080$) OF FRESH UNSTAINED PREPARATION OF BLOOD

The individual red cells in citrated isotonic platelet reticulocyte solution are well seen Cup-shaped cells are slightly thicker than normal. The full blown spherocytes in this preparation continue to show a small dimple.

FIG 24 PHOTOMICROGRAPH ($\times 1080$) OF NORMAL ROULEAUX FORMATION IN A FRESH UNSTAINED PREPARATION OF BLOOD MADE BY DROPPING A COVERSLIP CONTAINING A DROP OF BLOOD ON A GLASS SLIDE

The rouleaux gather together in long groups like piles of coins. The individual red cells can often be viewed on edge and one notes their regularity and their close 'fit' to each other

FIG 25 CASE 4 PHOTOMICROGRAPH ($\times 1080$) OF ROULEAUX FORMATION IN A FRESH UNSTAINED PREPARATION OF BLOOD FROM A CASE OF HEMOLYTIC ANEMIA

Note short rouleaux made up of red cells of varying grades of thickness and size The thick red cell must somehow accommodate itself to its neighbor Because of this, the rouleaux are usually of irregular shapes. A spherocyte frequently tops off a short rouleaux

FIG 26 F C PHOTOMICROGRAPH OF UNSTAINED PREPARATION OF BLOOD FOR DEMONSTRATION OF ROULEAUX FORMATION IN A CASE OF ACUTE HEMOLYTIC ANEMIA WHICH DEVELOPED IN THE COURSE OF A HITHERTO UNRECOGNIZED CASE OF CHRONIC LYMPHATIC LEUKEMIA

Note the small distorted rouleaux made up of red cells of varying degrees of thickness. The colorless cells are small lymphocytes

comes elongated into a jug-shaped cell, in which a "dimple" is seen at one pole. Finally, the dimple disappears and a completely round cell is formed. Examination of rouleaux formation is of great interest and is best made in fresh unmodified blood smears. Normal red cells form long rouleaux consisting of cells agglutinated together like a

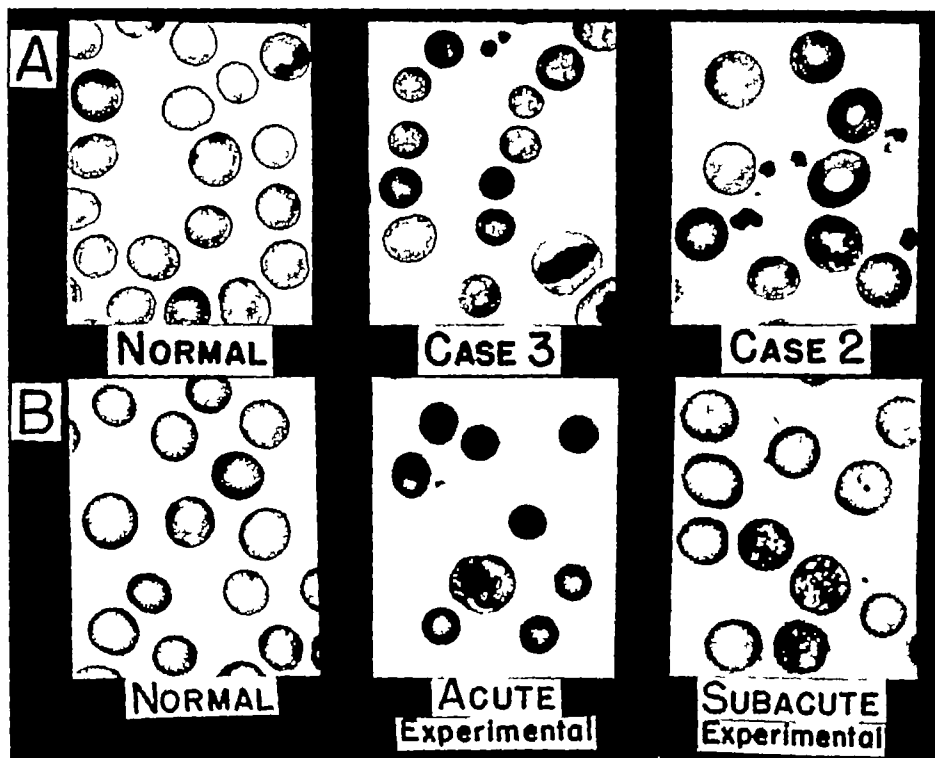


FIG 27 CORRELATION OF EXPERIMENTAL WITH CLINICAL HEMOLYTIC SYNDROMES

Both the experimental acute hemolytic anemia and the clinical fulminating case show very marked spherocytosis and but little reticulocytosis. In the subacute cases, both clinical and experimental, the reticulocytes and the normal red cells combine to form a "pseudo-macrocytic" blood picture resembling superficially that of pernicious anemia.

pile of coins (Fig 24). The red cells are relatively narrow and comparison of their thickness with the diameter of the free cells in the preparation will demonstrate a thickness/diameter ratio of approximately .25. Spherocytic red cells, with their various gradations in morphology, form very abnormal rouleaux, which are characterized by the relative fewness of cells, their increased thickness, and the bizarre formations which develop (Figs 22, 30). The adherence of

cup-shaped cells to jug-shaped and normal red cells results in irregular, peculiarly shaped rouleaux which are quite characteristic (67a) When many spherocytes are present, the mean corpuscular thickness becomes definitely increased (see under Fragility)

Although the spherocyte was first described as such by Naegeli, references to cup shaped and spherical red cells appeared almost a century ago Thus Dujardin (86) in 1842 found many corpuscles shaped "like cups, or cupules (acorn cups) with thick borders" in blood altered by the action of sodium phosphate Dekhuyzen (76) in 1899 discussed "cup-shaped" erythrocytes which he found as a transient stage in mammals Hamburger (143), and Brinkman and Van Dam (31) found that red cells often appeared spherical in fresh preparations of blood upon which a cover slip had been placed Ponder (276) studied this phenomenon in extenso F T Lewis (207, 208) observed the varying changes in the red blood cells thoroughly and the illustrations in his Lewis and Stöhr's Histology (2nd ed, p 193) are excellent (208) Naegeli (245) first suggested that the spherocyte was *pathognomonic* of congenital hemolytic icterus which could thus be designated as "spherocyte anemia" or "globe-cell anemia" Gänsslen (114) made much the same observations and correlated the spherocytosis with the increased fragility Microcytosis had previously been described by Chauffard (43 et seq) and numerous other observers (10, 250, 102) Von Boros was the first to make careful studies of the increased thickness of the microcytes and showed that, although the cells were diminished in diameter, the cell volume was nevertheless normal This could only be explained by increased thickness Von Boros (341, 342) also proposed a "thickness index" to indicate the degree of change in thickness Haden (132, 133, 134) studied these phenomena further and concluded that "Naegeli's conception of microspherocytosis as the fundamental and probably constant inborn error in this disease (congenital hemolytic icterus) seems the correct one." He stated that "the shape of the red cell indirectly represents an anatomic variation from normal," in common with the "tower skull" and other abnormalities. Gänsslen (114), Naegeli (245) and Haden (133, 134, 135, 136, 137) all concluded that the microspherocytosis indicated a definite bone marrow defect and was pathognomonic of the disease. Thompson (327) stated "The spherical microcytes

are as pathognomonic of this disease as are the sickle cells, in sickle-cell anemia." On the other hand, von Boros (341, 342) and Heilmeyer (154, 155, 156) stated that they had observed the spherocyte in various conditions other than congenital hemolytic icterus. Heilmeyer and Albus (157) in 1935 reported 3 cases of acquired hemolytic icterus in which typical spherocytic red cells with altered fragility were present. Following splenectomy, there was complete disappearance of these features. In the following year Heilmeyer made a careful analysis of the hematological data of his cases and concluded that the increased diameter, diminished thickness, diminished thickness diameter ratio, and diminished fragility which occurred after splenectomy gave definite evidence that spherocytosis was not an inborn constitutional change but rather an expression of pathological splenic function. He concluded that the spleen acts upon erythrocytes in certain cases with resultant spherocytosis.

Our own experience, like that of Heilmeyer, both in clinical and in experimental cases, is directly contrary to the view that spherocytosis is primarily due to a bone-marrow defect or is pathognomonic of the congenital form of hemolytic icterus. We were led to the view that spherocytosis might be due to the activity of a hemolytic agent in the serum by the findings in our third case of acute hemolytic anemia, which was characterized by the presence of large numbers of spherocytes and greatly increased fragility. In this case, as the titer of hemolysin diminished, the red cell diameter increased and the erythrocyte fragility became normal. Similar cases had previously been described by Chauffard, Troisier and Girard (49), and by Widal and Weissenbach (369). In previous studies of Henstell and Dameshek (158) of the bone-marrow and blood in a case of congenital hemolytic icterus, it was noted that the red cell diameter of the most mature nucleated red cells in the bone-marrow averaged 9.84 micra (normal or slightly increased), whereas the average red cell diameter of the peripheral blood was reduced to 6.76 micra. Furthermore, the most mature nucleated red cells in the peripheral blood of the clinical case of acute hemolytic anemia above referred to were of normal diameter, 8.9 micra, as compared with the average cell diameter of the non-reticulocyte red cells of the same day 6.85 micra. The average cell diameter of the reticulocytes in this case was 8.16 micra, whereas

that of the non-reticulocytes (mature red cells) was reduced to 6.18 micra. These clinical findings, demonstrating that the immature red cells of the bone-marrow and peripheral blood, were of normal size while the mature red cells were distinctly smaller than normal, could only indicate that the spherocytes were formed not in the marrow but by the action of some agent on circulating red blood cells. Support of these views was given in our animal experimentation.

In the experimental hemolytic syndromes the number of spherocytes varied directly with the dosage of hemolytic serum, i.e., the

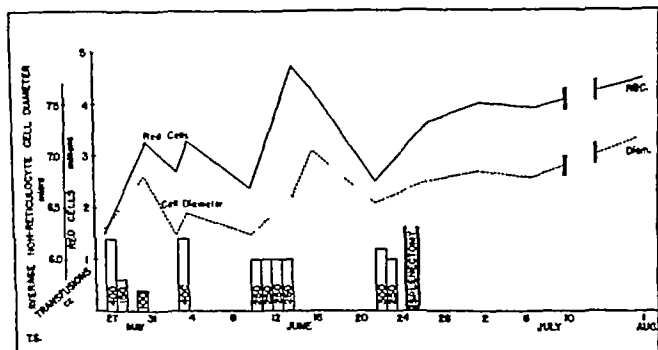


FIG 28 CASE 4 CORRELATION OF ERYTHROCYTE COUNTS AND THE AVERAGE DIAMETER OF THE NON RETICULOCYTE RED CELLS

With increasing hemolysis and dropping erythrocyte count the number of spherocytes increased and the cell diameter diminished. Following splenectomy the cell diameter gradually returned to normal levels.

larger the dosage, the greater the number of spherocytes. The Price Jones curves brought out graphically the changes which developed in the mature circulating red cells as hemolysis took place (Figs 29-32). These changes have been discussed in detail above. The nucleated red cells of the peripheral blood were always normal in size and nucleated microcytes were never encountered. Studies of the red cell diameters of the most mature nucleated red cells of the bone-marrow in animals dying with almost total microspherocytosis of the peripheral blood, gave normal values for the bone marrow cells. In an animal dying of acute fulminating hemolytic anemia, for in

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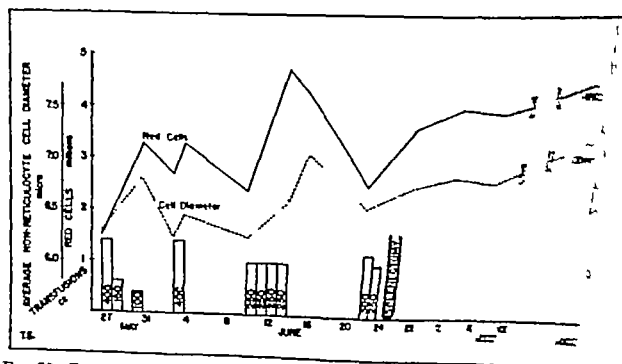


FIG 28. CASE 4 CORRELATION OF ERYTHROCYTE COUNTS AND AVERAGE DIAMETER OF THE NON-RETICULOCYTE RED CELL

With increasing hemolysis and dropping erythrocyte count the average diameter increased and the cell diameter diminished. Following cessation of the hemolysin the values gradually returned to normal levels.

larger the dosage, the greater the number of spherocytes. The Price Jones curves brought out graphically the changes which developed in the mature circulating red cells as hemolysis advanced (Figs 29-32). These changes have been observed in fatal cases. The nucleated red cells of the peripheral blood were always normal in size and nucleated microcytes were never encountered. Studies of the red cell diameters of the most mature nucleated red cells of the bone marrow in animals dying with acute fatal hemolytic anemia of the peripheral blood, gave normal values in the bone marrow. In an animal dying of acute fulminating hemolytic anemia the

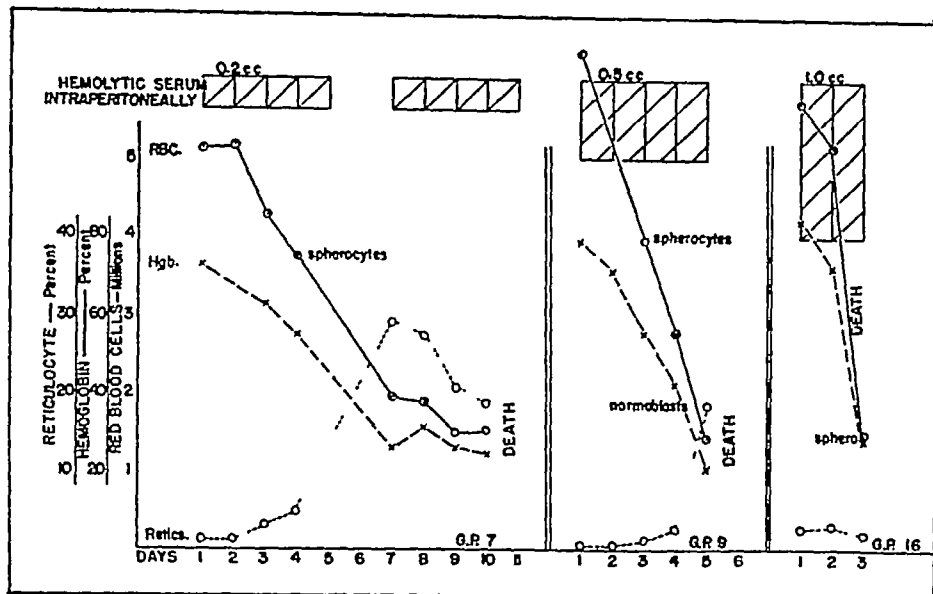


FIG 29 CORRELATION OF EXPERIMENTAL WITH CLINICAL HEMOLYTIC SYNDROMES
The type of syndrome produced experimentally in guinea pigs by the injection of hemolytic serum varies directly with the dose. A relatively small dose produces a sub-acute syndrome with spherocytosis, and secondary reticulocytosis, a moderately large dose produces marked spherocytosis with only abortive reticulocytosis, a large dose results in quick death with hemoglobinuria.

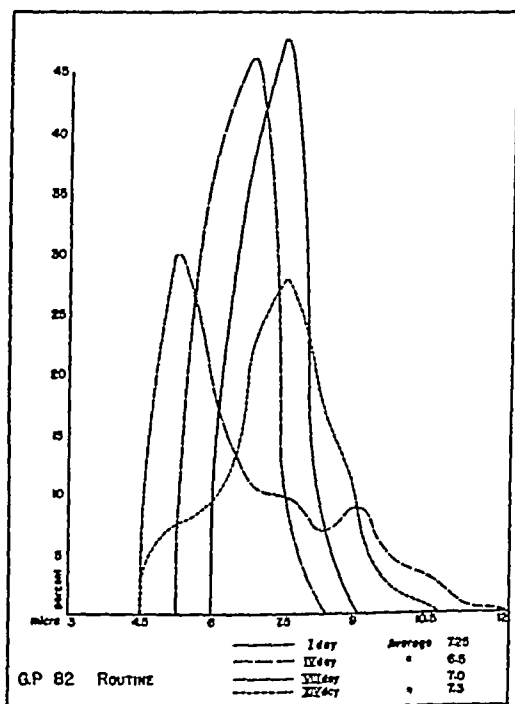


FIG 30 CORRELATION OF EXPERIMENTAL WITH CLINICAL HEMOLYTIC SYNDROMES

Price-Jones curves (total number of red cells, both reticulocytes and non-reticulocytes) in the guinea pig. Following injection of hemolytic serum, the red cell population becomes smaller, then definitely spherocytic (7th day) and then more normal with recovery. The wide, double-humped curve of the 7th day is analyzed in the following figure.

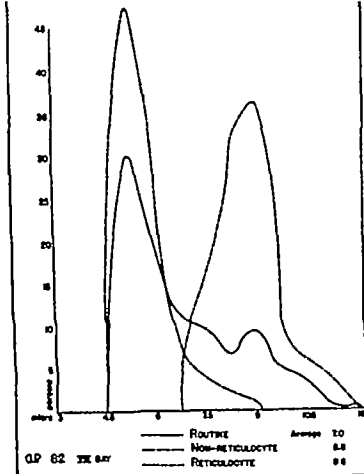


FIG 31 CORRELATION OF EXPERIMENTAL WITH CLINICAL HEMOLYTIC SYNDROMES

Differential Price-Jones curves of the reticulocytes and non reticulocytes on the 7th day following injection. The double-bumped curve obtained when all the red cell diameters are counted is broken up into 2 totally different *regular* curves (1) of small (spherocytic) mature red cells and (2) of large (immature) reticulocytes. The marked similarity of these curves to those of T S of 5/27/38 (fig 20) analyzed in the same manner may be noted.

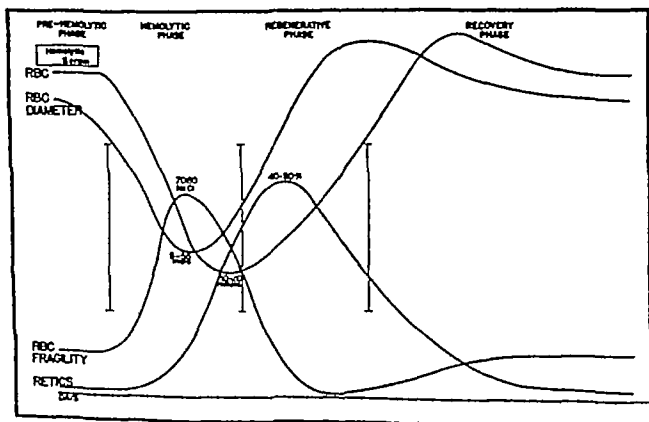


FIG 32. CORRELATION OF EXPERIMENTAL WITH CLINICAL HEMOLYTIC SYNDROMES

Diagrammatic presentation of the events which transpire in experimental acute hemolytic anemia. As the erythrocyte count diminishes, the red cell diameter (non reticulocytes) also diminishes rapidly. With increasing spherocytosis, the fragility of the red cells becomes greatly increased. This hemolytic phase is encroached upon by the rapid development of a regenerative phase during which there is marked reticulocytosis, in

stance, the average diameter of the most mature normoblasts was 9.3 micra (normal), whereas there was extreme microcytosis of the peripheral blood with an average cell diameter of 5.2 micra. We believe that these findings show conclusively that (a) *spherocytes are formed outside of the bone-marrow*, (b) *spherocytosis develops only in mature red cells*, (c) *a hemolytic agent, such as is present in hemolytic sera, is responsible for its development*. As a corollary to these views,

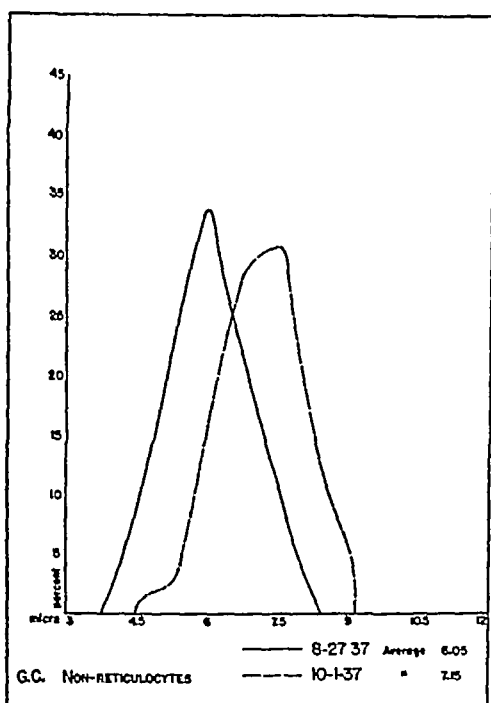


FIG. 33 CASE 3 PRICE-JONES CURVES OF NON-RETICULOCYTES (MATURE RED BLOOD CELLS) BEFORE AND AFTER SPLENECTOMY

Note the marked micro (sphero)-cytosis before operation and return to a more normal type of curve on 10/1/37. Spherocytosis in these cases occurs almost exclusively in the mature erythrocytes.

the concept that spherocytosis is pathognomonic of congenital hemolytic jaundice and indicative of an inherited bone-marrow defect is cast into question. We have been able to produce spherocytosis in animals by other hemolytic agents, such as distilled water and phenylhydrazine (67). Price-Jones (280), and Kammer and Rohnstein (176) also noted that microcytosis became prominent during the phase of hemolysis in experimental hemolytic anemia produced by the

latter drug Banti (10) noted microcytosis and increased fragility in animals given tuluoldiamine. In clinical reports of hemolytic anemia due to such agents as malaria and sulphanilamide, the microcytosis is pointed out, without much attention being paid to the phenomenon. For example, careful inspection of the photomicrograph in Harvey and Janeway's (148) recent article on acute hemolytic anemia due to sulphanilamide will reveal the presence of several typical spherocytes.

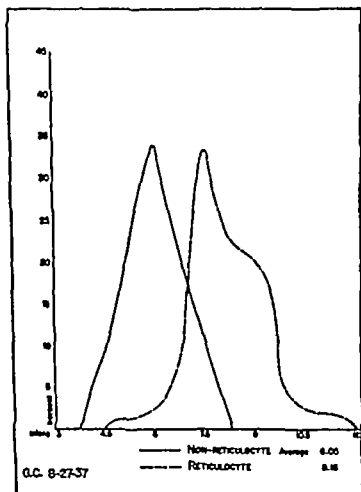


FIG 34 CASE 3 DIFFERENTIAL NON RETICULOCYTE AND RETICULOCYTE PRICE JONES CURVES 8/27/37

The non reticulocytes are practically all microcytes (spherocytes) and contrast sharply with the reticulocytes which are much larger and comprise many microcytes from 9 to 12 micra in diameter. From these and other data, it is probable that the microspherocytes are produced outside the marrow by an intravascular hemolytic agent.

We believe, therefore, that *spherocytosis* represents an alteration in the mature red cell brought about by various types of hemolytic agents, and that it is as indicative of increased hemolysis as *bilirubinemia*. In congenital hemolytic icterus, the spherocytosis may be due to the more or less continued action of an hemolysin. The extreme spherocytosis of the crisis of this disease may be due to the sudden liberation

of large amounts of hemolysin and the resultant action upon the mature red cell

2 *Evidences of increased blood formation, the pseudo-macrocytic blood picture* It may be stated as almost axiomatic that whenever increased blood destruction is present, evidences of increased blood formation always appear. These were abundantly present in our cases and included reticulocytosis, the presence of nucleated erythro-

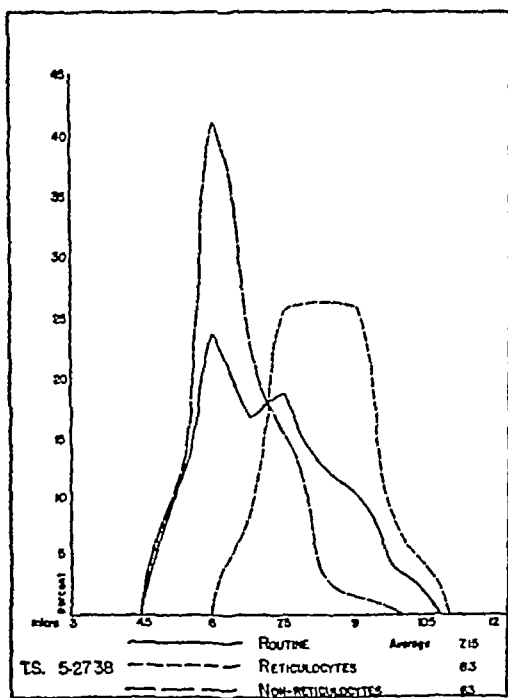


FIG 35 CASE 4 DIFFERENTIAL PRICE-JONES CURVES OF RED CELLS, RETICULOCYTES AND NON-RETICULOCYTES

The mature red cells (non-reticulocytes) are much smaller than the reticulocytes which are approximately normal in size

cytes, Howell-Jolly bodies, leukocytosis, the presence of myelocytes, etc. Of these phenomena, the greatly increased reticulocyte count was most interesting and is probably responsible for the frequently reiterated statement that the blood picture is "pernicious-anemia like" or "macrocytic." Our studies have shown that this is more apparent than real and might therefore, be called "pseudo-macrocytic." This is well brought out in differential Price-Jones curves of reticulocytes and non-reticulocytes (Fig 31). The average cell

diameter of the entire red cell population in a given case may be greater than normal, but analysis of reticulocyte and non-reticulocyte diameters demonstrates forcibly that the larger cells are almost all reticulocytes and that the non-reticulocytes are either normal or microcytic. Numerous investigations have shown that the relatively immature reticulocyte is larger than the mature erythrocyte and this also is brought out in our own studies. The "macrocytic" character of these cases of acute hemolytic anemia as stressed by Davidson (68, 213) and others may be due at least partly to the presence of large numbers of (polychromatophilic) reticulocytes and not to true mature orthochromatic macrocytes, such as are seen in pernicious anemia. This should be emphasized since it is an important feature in the differential diagnosis of the two diseases.

Watson (346 a) has recently criticized our views regarding "pseudomacrocytosis," emphasizing the macrocytic character of his cases of acquired hemolytic anemia as distinguished from those of the congenital variety. Unfortunately, actual measurements of the cell diameters were not made, only the mean diameter as determined with either the Bock erythrocytometer or the Pijper halometer being observed. In our hands the latter instruments have proved unreliable and of course give no data regarding the *varying* diameters of the red cell population. Setting this matter aside, however, it should be noted that the apparently macrocytic picture of at least most cases of "macrocytic" hemolytic anemia is produced by a combination of polychromatophilic reticulocytes with mature normocytes, spherocytosis usually being slight. Cases 1 and 2 of our series would have been called "macrocytic" by most observers and yet differed from cases of pernicious anemia by the lack of true *orthochromatic* (mature red cell) macrocytosis. These points are brought out in the Price-Jones curves of Case 2 and of guinea-pig 82 (figures 12, 30, 31). The mean corpuscular volume in Case 2, although definitely elevated at 125 cu micra, was associated with a normal mean cell diameter (Price-Jones) of 7.44 micra. The most immature reticulocytes averaged 8.70 micra, the mature erythrocytes averaged 7.15 micra. Even with a definite increase in mean cell volume, there may be an increased fragility of the red cells, perhaps due to their increased thickness. This was present in two of Watson's cases and in 5 of the 6 cases (all

chronic examples of acquired hemolytic icterus) reported by Dyke and Young (87 a) (Also compare Kremer and Mason (183 a)) It cannot be denied that certain cases in which true macrocytosis is commonly present (cirrhosis of the liver, "splenic anemia," and reticuloendotheliosis) may present hemolytic features in association with true orthochromatic macrocytosis Such cases are used by Watson (P 1787 of his article, Cases 5, 6, and 7) to illustrate his criticism of our viewpoint of "pseudomacrocytosis" in acute hemolytic anemia We believe that these cases represent a type of acquired hemolytic anemia which should be distinguished from the idiopathic variety considered in this paper One must conclude that the question of "macrocytic" hemolytic anemia should be kept *sub judice* at the present time

Nucleated red blood cells are common, particularly in the fulminating cases, and may be so numerous as to cause an apparent increase in the leukocyte count The latter figure should in this event be adjusted The nucleated cells are always normoblasts, with nuclei composed of thick "lumpy" masses of chromatin Megaloblasts of the true liver extract deficiency type (64) are never seen, although many authors have stated that they are present⁷ The "scroll-work" nucleus of the megaloblast is quite distinctive and readily differentiated, once it is known, from the nucleus of the normoblast The presence of many nucleated red cells in a case of supposed pernicious anemia should always cause one to suspect the possibility of hemolytic anemia, since contrary to common opinion, nucleated red cells in any great number are unusual in pernicious anemia

Other evidences of increased regenerative activity which are commonly seen are Howell-Jolly bodies, Cabot ring bodies, and basophilic stippling The large number of polychromatophilic cells of various shades of bluish-gray has already been commented upon

The leukocyte picture probably varies with the duration of the process In the first stage of the disease, the count is almost always very high, although in occasional cases the count is low from the start Later, the count may become quite low This is well brought

⁷ We use the term megaloblast in its original sense as a cell seen only in the embryonic state or clinically in the bone-marrow of pernicious anemia Morphologically, it may be sharply distinguished from the normoblast.

out in the case of Chauffard and Vincent (50) in which the initial white count was 40,000, soon dropping to 9000 and later (in three weeks) dropping to 2400 per cu mm. The differential blood picture is characterized by immaturity of the granulocytes with the presence of varying numbers of myelocytes. This changing blood picture may be explained in this manner: at first, with intense bone marrow regenerative activity, the entire marrow participates and the leukocyte count becomes greatly elevated, with continued destruction of red blood cells (and therefore continued stimulation to formation) the leukocyte production in the marrow tends to be interfered with. This was actually visualized in our bone marrow biopsies which showed extreme normoblastic hyperplasia with marked preponderance of nucleated red blood cells over whites (about 3:1, normal about 1:1). The low leukocyte counts of some cases at the beginning of the disease may be due to some other mechanism such as "depression" of growth or "maturation arrest," perhaps due to splenic activity.

The blood platelet picture probably has similar physiological background. At the beginning, with intense regenerative activity, the entire marrow participates and thrombocytosis is present, but later as megakaryocytic tissue is probably overrun by erythroblastic, the platelet count may diminish to fairly low levels.

c. *The blood picture during recovery* The blood picture during recovery may roughly be divided into three stages: (1) the immediate, (2) the later or erythroblastic, (3) the final stage of complete recovery (Figs 18, 19, 20, 21). The immediate effect of splenectomy was exceedingly dramatic in all the cases and was followed by an almost instant rise in erythrocyte count and hemoglobin without reference to previous transfusions of blood. This phase lasted from 4 to 7 days during which time the patient's clinical condition improved remarkably, and the erythrocyte count rose on the average of 200,000 to 300,000 per day. The first stage was followed in our cases by a second stage characterized by perceptible lag in erythrocyte increase, during which marked erythroblastosis was the rule. The blood picture at this time was characterized, particularly in Cases 2 and 3, by the presence of large numbers of normoblasts in varying stages of maturity, by marked reticulocytosis, and by the presence of large numbers of Howell-Jolly bodies in the erythrocytes. Nuclear frag-

ments and stippled red blood cells were also present at this time. Despite these marked evidences of regenerative activity on the part of the erythroblastic tissue, there was very little rise in erythrocyte count. Even with transfusions this type of blood picture continued and since the possibility that there might be a faulty regenerative effect or loss of maturation substances in the bone-marrow, liver extract and iron were given in Cases 1, 2 and 3 following which gradual improvement occurred. Whether due to transfusions, to the added effects of iron and liver extract, or to other mechanisms, this second stage of recovery gradually gave way in the course of 4 to 6 weeks to the final stage of complete recovery during which the reticulocytes dropped to normal, the icterus index fell to normal, the urobilinogen excretion became normal, and there was a gradually increasing rise in hemoglobin and red cell count. Within three months, normal counts were reestablished. In Cases 1, 2, and 3 (Figs 18, 19, 20) a slight though definite increase in reticulocytes took place approximately a month after splenectomy. (The fourth case was not observed sufficiently long at daily intervals to note this effect.) This secondary reticulocyte response without relation to liver extract or transfusions might have been due to the stimulating effect upon the bone-marrow of the breakdown of the large numbers of foreign red blood cells which had been suddenly introduced into the body just prior to splenectomy and about a month previously.

This analysis of the blood picture in recovery is taken from our own cases which, it must be admitted, were of great severity, necessitating splenectomy. Fiessinger et al (109) speak of the anemia as being of the "*coupe d'archet type*"—i.e. like an arc with rapid lysis followed by rapid recovery. This is certainly the case, particularly in some children where exceedingly rapid blood destruction is followed, either spontaneously or after transfusion, by extremely rapid recovery. Analysis of many of the cases will, however, demonstrate that a definite lag in recovery, comparable to our second stage described above, was frequently seen. In the series of Lederer (197, 198), who speaks of the dramatic response to transfusion, a definite and marked lag in rise was discernible in Case 3 of series 1 and in Case 1 of series 2.

The interpretation of these stages cannot be entirely worked out in the present state of our knowledge. The immediate effect of splenectomy may be due to the sudden removal of an organ (1) which in-

hibits red blood cell formation in the marrow, (2) destroys large quantities of weakened red blood cells, (3) produces hemolysins. The second stage of "futile" red cell formation may be due to poor or inefficient maturation, or to combinations of these factors. The final stage of recovery is of course due to the loss of continued hemolysis and the associated resumption of normal marrow activity.

d *Fragility of the red cells* The resistance of the red cells to hypotonic salt solutions was normal in Cases 1 and 2 of our series, greatly diminished in Case 3, and moderately diminished in Case 4. In Case 3, the red cells were extremely fragile upon the patient's admission to the hospital, but with her continued recovery, their fragility finally became entirely normal. This coincided with an increasing red cell diameter and gradual diminution of spherocytic microcytes, and with a diminishing titer of hemolysins.

The first two cases with normal saline fragility were subacute, with many normal-sized and large usually polychromatophilic red cells present, quite in contrast with the appearance of the second two cases which showed a good deal of spherocytosis. That the fragility tests should be normal in the presence of a hemolytic process with definite numbers of spherocytes present is rather puzzling. In the presence of large numbers of spherocytes as in Cases 3 and 4, the fragility test is grossly abnormal. The same situation was found in the experimental animals above referred to. When the blood picture became "pseudo-macrocytic" the fragility test became normal or relatively so. This is quite in contrast with the findings in congenital hemolytic icterus, in which, following splenectomy, the fragility test may retain its abnormality, despite the presence of relatively few spherocytes. This brings up the possibility that in the latter disease, as noted by Vaughan (339), the increased fragility may not entirely be due to spherocytosis. Certain studies in "differential fragility" (67)—(i.e. using different types of hemolysins against the same erythrocytes) may help to explain the apparent discrepancies by indicating that spherocytes from different conditions, although morphologically identical, may have physiological differences. At any rate, one can say quite definitely that the more acute the process, the more marked is the fragility, as the process becomes subacute and relatively prolonged in the acquired form of the disease, the saline fragility tends

to be relatively normal In many of the reported cases, readings of beginning hemolysis at 50–56 NaCl have been found

Hamburger (143) showed that by the use of varying dilutions of sodium chloride with water, gradations in erythrocyte fragility could be determined Chauffard (43) was the first to put this laboratory finding to important clinical use when he demonstrated that the red cells of the hereditary form of hemolytic jaundice were abnormally fragile Widal and his collaborators (361) pointed out that it was advisable to use washed red cells in the test, particularly with the acquired cases, in which the erythrocyte fragility might be only slightly altered The abnormal fragility of both forms of hemolytic icterus (cf Eppinger (102)) was taken for granted until Naegeli's (245) insistence that spherocytosis and increased saline fragility were pathognomonic of the congenital type This concept gradually spread until finally such observers as Haden (132–134), Thompson (327), and many others accepted Naegeli's dictum that increased fragility was the *sine qua non* for the diagnosis of congenital hemolytic icterus, and conversely that this finding in any other condition was more apparent than real On the other hand, Meulengracht (229) stated quite definitely (1922) that increased saline fragility was not pathognomonic of congenital icterus and described cases of the acquired type with increased fragility Furthermore, he stated that this symptom might be considered either (1) as an inherited disturbance in formation (2) as a regeneration phenomenon or (3) *as the expression of the activity of toxic or hemolytic forces upon red cells in the circulation* This latter view, as noted above, agrees with our own

The correlation of spherocytosis and increased fragility has been commented upon by several groups of observers Thus Gansslen (114) in 1922 suggested that the spherical cells of congenital hemolytic icterus were more fragile because a sphere has the greatest volume for the least surface To reach osmotic equilibrium, the spherocyte will take up proportionately more water than the normal red blood cell, because of the already spherical form, however, it will be less capable of accommodating an increased quantity of fluid and will therefore burst Ponder (275–277), in a series of publications, showed that the differences in the erythrocytic fragilities of various animal species could be correlated with their cell shape Haden (132) in 1935 demonstrated the direct relationship between the spherocyte and

increased fragility and proved the dependency of resistance upon cellular thickness. He worked out a simple formula for thickness and demonstrated the linear correlation between thickness and cellular fragility. Castle and Daland (40) confirmed the work of Ponder and of Haden, and showed by direct microscopic observation that "(a) hemolysis of a given type of erythrocyte is associated with the assumption of a spherical form in hypotonic plasmas (b) the more susceptible the erythrocyte to hypotonic hemolysis, the less hypotonic is the plasma necessary to cause the assumption of a spherical form." In other words, these observers showed that significant differences in the strictly osmotic behavior of red cells did not exist, but that differences in erythrocyte fragility could be explained entirely by differences in the form of the red cells.

Haden and most other observers have considered that the more fragile spherocytes of congenital hemolytic icterus resulted from an improper formation of cells in the bone-marrow. However, a few observers (205, 339) have commented upon the fact that following splenectomy, the cellular population changes materially, and with it a diminished fragility ensues. That there is for the most part an exact correlation between spherocytosis and increased fragility cannot be denied, in fact it may be said that the degree of fragility is simply another method of expressing the degree of spherocytosis present. We feel, however, that spherocytosis (and increased fragility) is due, not to improper blood formation, but to the previous activity of a hemolytic factor. Troisier (332) concluded that fixation of hemolysin to the red cells, with their resultant sensitization was the cause of their increased fragility. Following the publication of Troisier's thesis (1910), Chauffard, Troisier, and Girard (49) in 1912 and Widal and Weissenbach (369) in 1913 described unusual cases in which not only was increased fragility present but free hemolysins were discovered in the serum. In both of these cases, as the patient recovered, the isolysins gradually became diminished and the fragility test became normal. These cases are quite comparable to Case 3 of our series in which severe anemia, spherocytosis, increased fragility, and high titer of hemolysin were present, but as the patient recovered, all the various factors became normal. Our own clinical and experimental observations have led us to the conclusion that increased fragility of the red cells is due to the previous action of various types of extrinsic factors upon mature non nucleated red cells.

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C Treatment

An analysis of the various methods used in the treatment of this disease and of their results has already been given above. It is the purpose of this chapter to discuss the proper utilization and rationale of these methods.

1 Transfusions a *Transfusion is not always successful* That transfusions are of distinct benefit in this condition has been abundantly shown. In fact the conception has gradually developed that a dramatic response to a single transfusion of blood is pathognomonic. Thus, if the patient fails to respond even to a single transfusion, the diagnosis of acute hemolytic anemia (so-called Lederer's type) is held in question, this is most certainly true if several transfusions have been given and the patient, although receiving temporary benefit, continues his downhill course. That some patients fail to respond quickly to one or two transfusions is brought out in several cases reported in the literature, as noted above. Although the discussion is apparently an academic one, it becomes exceedingly practical from the standpoint of further therapy when transfusions have been shown to be valueless. Thus, the patient's condition may become so precarious in the course of waiting for a favorable result to ensue from transfusions that the life-saving procedure of splenectomy might then be considered too dangerous to attempt. If a successful splenectomy is carried out in the wake of an unsuccessful response to transfusions, the criticism is made that one is dealing not with "Lederer's anemia" but with hemolytic icterus of an atypical, acquired type or possibly with an unrecognized example of congenital hemolytic icterus in crisis. Thus, in our first three cases one observer stated that they were examples of acute hemolytic icterus and not of acute hemolytic anemia! In Case 4, another observer who had himself previously diagnosed the condition as "acute hemolytic anemia of the Lederer's type" felt that the failure of response to repeated series of transfusions pointed to a hitherto unrecognized congenital disease now in crisis and requiring splenectomy.

Our own conceptions are completely at variance with these ideas. Some cases are so mild as to respond spontaneously, others may improve with a single transfusion, still others require several transfusions, and a certain and not altogether small, percentage of cases require the

more serious procedure of splenectomy as an emergency measure. The differences in response are, we feel, due simply to differences in severity of the disease, the most severe examples requiring the most drastic treatment. About the same situation holds true in thrombocytopenic purpura. Mild cases may respond to transfusions or to some other form of medical therapy, that splenectomy is required in certain more severe cases does not imply that one is dealing with a different disease. It is simply a difference in the degree of severity of disease.

b Rationale of transfusions Lederer does not state his reasons for utilizing transfusions in his first three cases, although since marked anemia of the apparently "pernicious" type was present, the procedure seemed a rational one. The response without sequelae was certainly dramatic and perhaps somewhat unexpected. Since the effect can only partially be explained on the basis of the slight increase in red cell count, the effect of introducing normal serum must naturally be discussed. Besredka (21) in 1903 demonstrated that normal human serum had an antilytic action and postulated a balance between lytic and antilytic elements in the serum. Clark and Evans (54) in 1920 also found that normal serum has a constant protective action but that in hemolytic anemias the protective action was markedly diminished. Josephs (172, 173) has recently extended these observations. He found that in cases of sickle cell anemia and of erythroblastic anemia there was a definite reduction of urobilin excretion following transfusion of either whole blood, blood plasma, or a serum extract. He interpreted this result as indicative of the presence of an antilytic agent in normal serum. Our own findings are interesting in this regard. In vitro studies of the hemolysis in Cases 2 and 3 demonstrated a marked antilytic activity of normal human serum.

Thus it is not at all unlikely that the sudden introduction into the body of normal human serum results in an interaction of normal antilyns with either free or cellular lysins resulting in either temporary or permanent benefit. The lack of effect of a single or several transfusions may readily be explained in this manner: i.e. either insufficient antilyns are introduced or there is continued lysis production with only temporary benefit from transfusion. In these cases, splenectomy is of value possibly because the chief lysis producing organ is removed or normal blood destruction is diminished. Further

studies await the isolation of an antilysin from the serum and its use in hemolytic anemia

c. *The question of reactions to transfusions and use of specific types*
In Case 3 of our series (Group B), a severe reaction with hemoglobinuria occurred when a transfusion was given from a Group O donor. Reactions did not occur at previous or later transfusions when Group B donors were used, but again occurred when another Group O donor was used. In this case the most likely explanation for the severe reactions probably lay in the use of a "universal donor" for a case with fragile red blood cells. Although Group O donors are theoretically always compatible because their red blood cells (without agglutinogens) are incapable of being agglutinated (or hemolyzed) by the serum of the prospective recipients, it must be remembered that their serum contains both alpha and beta agglutinins. Thus, when administering blood from a Group O donor to a patient with severe anemia, agglutination of a goodly proportion of the recipient's cells might conceivably occur, particularly if the titer of the donor's serum was somewhat higher than usual either in alpha or beta agglutinin (or hemolysin). This is particularly true when transfusions are to be given to a case in which the red cells are more fragile than normal. Here, the titer of agglutinins and hemolysins in the donor's serum might be just sufficient to cause agglutination or hemolysis of the recipient's relatively few and already more fragile red cells in the circulating blood.

Sharpe and Davis (313) have recently warned of the dangers of giving transfusions to patients with acute hemolytic crisis and acute hemolytic anemia, and even suggest that the procedure is too dangerous for general use. They report two cases in which severe reactions occurred when transfusions were given. Unfortunately, the very important question of the blood groups of the patients and donors concerned is not mentioned. This is the crux of the situation. In six transfusions given in Case 1, eight transfusions in Case 2, ten transfusions in Case 3, and twelve transfusions in Case 4, reactions occurred on only three occasions, once in Case 2 and twice in Case 3. In 3 recently observed cases of crisis occurring successively in three members of one family with congenital hemolytic jaundice, 3-5 transfusions were given in each case, again without reactions. Thus, not only was it *not* dangerous to transfuse these individuals, but the transfusions were of distinct, although temporary benefit, and an

absolute necessity as a pre-operative measure. This record was attained because the simple precaution was always observed (after our experience with Case 3 of this series) to use blood from a donor of the same type as the patient. Dependence upon simple tests for compatibility and the use of "universal donors" not infrequently lead to severe reactions. In severe anemia, particularly of the hemolytic type, in which fragile cells are usually present, we advocate the use of donors of the same type as the patient. When this is done, several transfusions can almost always be given with impunity.*

A rare cause for a transfusion reaction in cases of acute hemolytic anemia is the reverse mechanism, that is, the serum of the patient may have such a high titer of hemolysin that the red cells of certain donors will be hemolyzed. This was possibly the case in one transfusion reaction of Case 2, and might have been partially responsible for the reactions of Case 3. This possibility is usually guarded against by testing the prospective donors' red cells against the patient's serum in the ordinary way. Although this procedure is usually sufficient and indeed resulted in the discarding of many prospective donors in Case 2, it is possible that hemolysis may only occur after 1-2 hours of incubation. Failure to carry out the latter procedure may result in a delayed transfusion reaction. Thus, in a case of acute hemolytic anemia it is necessary (a) to use blood from the same blood group (b) to be certain that the patient's blood serum does not contain hemolysins effective against a prospective donor's blood cells. It is, therefore, recommended that both the slide and the test tube methods for testing compatibility be utilized and that cross-matching of serum vs cells of both recipient and donor be made. Furthermore, incubation of the patient's serum with the prospective (and compatible) donor's red cells *for one hour* should be performed before a final reading is made.

It should also be remembered that particularly when dealing with a patient of Blood Group A, new isolysins may be built up when several

* Since writing this chapter, we have observed three other cases of acute hemolytic anemia in which severe transfusion reactions occurred. In the first case, (Group A) given several transfusions of Group A blood, hemolysins for one of the subgroups of A were apparently built up, in the second case (Group O), death occurred when Group O blood was given. Since all the compatibility tests were negative, the explanation for this is not clear (? allergic reaction). In the third case the presence of a strong panagglutinin made typing almost impossible and transfusions very risky, one severe reaction resulted.

transfusions are given. Temporizing with too many transfusions, particularly in an elderly person with a vulnerable circulation, may not only result in a serious and perhaps fatal transfusion reaction but in overburdening the right side of the heart. Subsequent splenectomy may thereupon prove fatal. We believe therefore that if 3 or, at most 4, transfusions have not proved effective in controlling the disease, the operation of splenectomy should be performed.

2 Splenectomy The possible rationale for the use of splenectomy has already been discussed. Whether splenectomy simply removes the largest single blood destroying organ or the largest collection of hemolysin-producing cells or does both at once, there can be no question regarding the very effective results which are so quickly induced by this procedure. Our own cases show the lack of permanent effect of transfusions of blood and the dramatic and sustained effect of splenectomy with a return to a normal blood picture.⁹ To maintain that since splenectomy was necessary in these cases, they were not examples of acute hemolytic anemia (so-called Lederer type), seems absurd since all the various criteria were present except perhaps that of mildness of course. We feel that it is necessary to perform splenectomy (a) when it has been adequately demonstrated that transfusions are only of temporary benefit and (b) before the patient's reserves have been completely dissipated. In Case 4, only through the administration of four *series* of transfusions (12 in all) without permanent effect was it possible to convince various members of the family of the importance of doing splenectomy without further temporizing. In the first three cases, the patient's condition was either so critical or the disease so fulminating that further temporizing seemed inadvisable. Greenwald (126), in reporting two cases of acute hemolytic anemia, makes this statement: "Transfusion, while it acts as a specific in acute hemolytic anemia, is of little benefit and perhaps even harmful when given during a crisis to a patient suffering from acholuric jaundice. *Obviously, splenectomy, performed on a patient suffering from acute hemolytic anemia of the Lederer type, would be calamitous*" (italics ours). Greenwald does not state why this is so "obviously" the case, nor is this brought out in the body of his article. Emergency splenectomy is often a necessity in the acute hemolytic

⁹ Two cases have recently been observed in which splenectomy was of only transient (4-6 months) benefit. One of these cases subsequently recovered upon removal of a large dermoid cyst of an ovary.

crisis of the congenital cases As brought out above, no substantial difference exists between these cases and those with non congenital acute hemolytic anemia The treatment of both conditions should thus consist first of successive transfusions until the patient is either definitely improved or at least in better condition for splenectomy, this usually requires no more than 3 or 4 transfusions The patient should be watched minutely for we have ourselves seen drops of 1-3 million in red cell count within a period of several hours A case of acute hemolytic anemia (or crisis) is as much of a medical emergency as one of diabetic coma, and the physician should be alive to the possibilities of rapid hemolysis, possible transfusion reactions, and the possible need for emergency splenectomy It is only through knowledge of the seriousness of the situation that it can be kept well in hand and dangerous sequelae avoided Acute hemolytic anemia is not the mild disease which it has often been depicted As we have brought out above, several cases have died because this was not realized The summary disregard of splenectomy as a therapeutic measure has in some cases, to use Greenwald's word, proved "calamitous"

In the actual performance of the operation a surgeon should be selected who is both able and cognizant of the necessity for quick removal of the spleen Surgeons seem to differ greatly in their ability to isolate the organ and to remove it expeditiously Although rapid surgery is nowadays rather frowned upon, there can be no doubt that in this condition (and in fulminating thrombocytopenic purpura) speed is frequently of the essence *

D Classification of the hemolytic syndromes

For many years, the various hemolytic syndromes have been classified as separate entities, the possibility of their relationship either aetiologically or nosologically having only rarely been discussed With increasing knowledge of the physiology and pathology of hemolysis, this complete and unrelated separation should not long continue Rapid "deglobulization" may result in severe anemia, in which case

* Our recent experience indicates that splenectomy may be a very serious operation, particularly in the elderly patient. If 3 or 4 transfusions have proved ineffective, one is faced with the necessity of balancing the danger of the operation against the otherwise almost certain fatal termination To continue transfusions will usually result in reactions and an overburdening of the circulation

been described by Chauffard and his collaborators. It has been the custom in recent years to recognize only the first type, the "idiopathic" and "hemolytic" varieties having either been forgotten since 1915 or their presence doubted. Dawson (70), Meulengracht (228), Tileston (330), and following them, many other writers have either doubted or arbitrarily dismissed the acquired variety, although the concept of "Lederer's anemia" (merely an acute form of acquired hemolytic icterus) has been enthusiastically received. In recent years, the classification offered by Thompson and Whipple of "typical" and "atypical" hemolytic jaundice has gained ground. The "typical" cases present a chronic history and acute exacerbations, spherocytic anemia, splenomegaly, prompt response to splenectomy, and persistence of spherocytosis after the operation. The "atypical" group, admittedly heterogeneous, does not present spherocytosis or increased fragility, an etiological factor may or may not be demonstrated, and splenectomy is said to be of no value. According to these criteria, a case with spherocytosis, even if an etiological factor is demonstrated (such as leukemia) must be considered to be "typical." A "typical" case which does not respond to splenectomy (we have observed 3 such cases), or one in which the spherocytosis and abnormal fragility completely disappear after operation (Cases 3 and 4 in our series) cannot be pigeon-holed. The response of "atypical" cases (Cases 1 and 2 in our series) to splenectomy has been very gratifying. We believe, therefore, that the classification "typical" and "atypical" is of little significance and tends to becloud the issues which have been raised. The words "congenital" and "acquired," on the other hand, have readily grasped meanings without any underlying implications such as the necessity for a certain blood picture or type of response to splenectomy. We see no reason therefore for dropping the designation of acquired hemolytic icterus (anemia). Our classification is thus

- A Congenital hemolytic icterus (anemia)
 - 1 Chronic, with or without crisis
 - 2 Subacute
 - 3 Acute (crisis)
- B Acquired hemolytic icterus (anemia)
 - 1 Secondary to known cause (infectious, chemical, "toxic" (pregnancy, etc))

- 2 Symptomatic, in association with certain, usually malignant diseases, as lymphatic leukemia, Hodgkin's disease, carcinomatosis
- 3 Of unknown cause, with or without hemolysins in the serum.
 - a. Chronic, with or without crisis
 - b Subacute
 - c. Acute
 - d Acute fulminating, often with hemoglobinuria

Our studies have abundantly demonstrated that the picture of spherocytosis, increased fragility, reticulocytosis, and response to splenectomy is not pathognomonic of the congenital variety, but may be seen as well in the acquired type and may be reproduced experimentally. Spherocytosis (and increased fragility) are, we believe, the results of a reaction upon the mature red blood cell of a lytic agent. The contention that spherocytosis must of necessity indicate hereditary or congenital disease should be discarded.

SUMMARY AND CONCLUSIONS

1 An analysis is made of the cases of acute, acquired hemolytic anemia, based upon personal observation and a survey of the literature. Particular reference is made to the subject of the possible rôle of hemolysins

2 Serum iso-hemolysins, at first of high titer, were repeatedly demonstrated in 2 of our first 4 cases. A unique feature of the hemolysin was its ability to hemolyze cells of Group O and of its own blood group

3 Transfusions of blood were of only temporary benefit in our cases, but splenectomy was followed by prompt recovery. Of the 106 cases reported in the literature, transfusions were given in 66. In 22 of the latter group (one third), they proved unsuccessful in arresting the disease. Splenectomy was curative in 20 of 23 cases in which this operation was performed

4 The spleen showed one of three types of lesions: (1) multiple areas of thrombosis and infarction, (2) histiocytic proliferation often with erythrophagocytosis and giant cell formation, (3) congestion of the pulp. The bone marrow showed extreme normoblastic hyperplasia.

5 A critical review of the literature indicates that acute, non-congenital (acquired) hemolytic icterus (anemia) was first described by Widal, Abrami and Brulé and by Chauffard and his collaborators in 1901-1908. The latter described cases ("hemolytic icterus") in which hemolysins were present in the serum.

6 The interrelationships of bilirubinemia, bilirubinemia with anemia, hemoglobinemia, and hemoglobinuria are discussed. In hemoglobinuria there is such violent hemolysis that the immediate products of hemolysis cannot be converted to bilirubin.

7 At least four, possibly five different types of iso-hemolysins have been described in the various hemolytic syndromes. Similar syndromes were experimentally reproduced by the use of a hemolytic serum immunologically comparable to that found in our clinical cases. It is thus not improbable that hemolysins of various types and "dosages" are responsible for the various types of hemolytic diseases.

8 Studies of the bone-marrow and peripheral blood, together with the experimental evidence, show that spherocytosis and increased erythrocyte fragility are the end-results of the activity of hemolysins upon mature red blood cells and not due to faulty production by an abnormal marrow.

9 The so-called macrocytic picture of certain cases of acute hemolytic anemia may be due to the presence of large numbers of polychromatophilic macrocytes (reticulocytes) quite distinct from the mature orthochromatic macrocytes of pernicious anemia. The bone-marrow is furthermore normoblastic, with none of the features of the megaloblastic bone-marrow of pernicious anemia. Spherocytes are almost always present in the acute cases, their number depending upon the severity of the case (i.e., the rapidity of lysis).

10 Acute hemolytic anemia is not the benign disease which it is usually depicted, nor are transfusions always curative. Failure to recognize this may result in fatal termination. The possibility of splenectomy as an emergency procedure should always be kept in mind and operation should not be delayed following the demonstration that transfusions have been of only transient benefit. The almost immediate therapeutic effect of splenectomy in many cases is one of the most dramatic events in medical practice.

11 The beneficial effects of transfusion may be due in part to an

anti-hemolytic effect of normal serum and not entirely to an increase in the red cell content of the blood

12 The various hemolytic syndromes, both congenital and acquired, may be classified as fulminating (with hemoglobinuria), acute, sub-acute, and chronic Spherocytosis and increased fragility may occur in both the congenital and acquired varieties "Acute hemolytic anemia" is the acute variety of acquired hemolytic icterus (anemia) This type is at times associated with the presence of hemolysins in the serum ("hemolysin icterus") The terms "hemolytic icterus" and "hemolytic anemia" are interchangeable Because of the many prior descriptions, the eponym "Lederer's" anemia appears to be unwarranted

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PLASMA PROTHROMBIN, VITAMIN K¹

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In the past few years there has been a rapid increase in the knowledge concerning plasma prothrombin. These newer developments have resulted from work in many different, and often widely separated, fields, including physiology, chemistry, pathology, nutrition and therapeutics. Of particular significance are (1) the development of methods for the quantitative determination of prothrombin, (2) the discovery of the relation of the liver and a new accessory food factor, so-called vitamin K, to prothrombin formation, (3) the isolation and chemical identification of the new vitamin and (4) the application of these findings to the study and control of certain hemorrhagic diseases. In this review, an attempt has been made to summarize only those recent developments which relate to prothrombin and to vitamin K. The older literature on prothrombin has been summarized in the extensive reviews of blood clotting by Wohlsch (262), Howell (137) and Eagle (93).

PLASMA PROTHROMBIN PHYSIOLOGICAL CONSIDERATIONS

Role of prothrombin in blood clotting

The coagulation of blood involves a series of complex chemical reactions in which a number of clotting factors are concerned. These factors include prothrombin, thromboplastin, calcium, thrombin, and fibrinogen, as well as a group of clotting inhibitors, among which are plasma antithrombin, plasma antiprothrombin, and heparin. To this list one might add a number of other clotting substances which have been postulated by various workers from time to time, but none of which have been widely accepted.

Practically all workers in the field today adhere to one form or another of the thrombin theory of clotting, that is, the plasma protein, fibrinogen, is transformed into the fibrin clot by the action of

thrombin Thrombin is not preformed in the body, but develops only under special circumstances, as in shed blood, by the interaction of at least three substances calcium, prothrombin, and thromboplastin The calcium and prothrombin are both normally present in blood plasma, while thromboplastin, it is believed, is furnished only by injured tissues and by disintegration of the formed elements of the blood, especially the platelets Clotting thus can be designated as a two-stage process

(1) prothrombin + calcium + thromboplastin \longrightarrow thrombin

(2) thrombin + fibrinogen \longrightarrow fibrin (clot)

The thrombin theory of clotting implies that prothrombin is an indispensable factor in the coagulation process Although this was indicated by the older experiments of Morawitz and of Fuld and Spiro in 1904, this view, for several reasons, was not accepted by a number of investigators Loeb (lit by Silberberg (219)) and Mills and co-workers (176, 178) in particular have reported experiments which suggested that a tissue factor ("tissue coagulin" of Loeb, "tissue fibrinogen" of Mills) along with calcium would clot plasma fibrinogen directly, without the intervention of prothrombin While not denying the existence of the thrombin type of clotting, a second clotting mechanism was postulated—a type not requiring the presence of prothrombin

Recently several workers have investigated the "tissue fibrinogen" clotting of Mills, but they have not been able to substantiate the finding of a one stage clotting mechanism Smith, Warner and Brinkhous (220) found that clotting in a mixture of "tissue fibrinogen" (lung extract), calcium and fibrinogen was due to the presence of contaminating prothrombin in the plasma fibrinogen or in the "tissue fibrinogen" In their experiments, the lung from which "tissue fibrinogen" is prepared was first thoroughly perfused to remove all the blood, hence the plasma prothrombin The plasma fibrinogen was obtained free of prothrombin by an adsorption technique On adding calcium to a mixture of these two preparations, no clotting occurred They concluded that the "tissue fibrinogen" is merely a highly potent form of thromboplastin which contains traces of prothrombin, unless special precautions are taken to remove the latter

Quick (194) and Davison (87) have also investigated this problem, and have come to the same conclusion

Fuchs (118) and Fischer (112) believe that prothrombin is a constituent, not only of plasma, but also of certain tissue proteins, especially of skeletal muscle. Thus, tissue prothrombin presumably could substitute for plasma prothrombin in clotting. The data given above conclusively indicate that lung tissue freed of blood contains no demonstrable prothrombin. Howell (137) has pointed out that residual blood may account also for the prothrombin found in muscle extracts by Fuchs and by Fischer. Also, their test reagents may not have been completely freed of prothrombin. Ferguson (98) used corneal and lens extracts to avoid the first of these difficulties. Using carefully prepared fibrinogen, the eye extracts produced, with calcium, a very slow type of clotting (as long as $1\frac{1}{2}$ days). The significance of such long clotting times may be questioned, in view of the fact that a prothrombin concentration of less than 0.01 per cent of that of plasma is sufficient to give clotting in a few minutes (45). Thus, the minute amounts of prothrombin which must have been present in Ferguson's experiments may well have resulted from diffusion of prothrombin from the plasma or lymph, as he has pointed out (98), or possibly from contamination of fibrinogen or tissue with plasma prothrombin, in spite of the precautions taken.

The present evidence indicates that prothrombin available for clotting is present with certainty only in plasma (and lymph). Also, the necessity of prothrombin for clot formation appears to be well established. Views to the contrary would seem to be due to difficulty in obtaining the clotting factors in pure form.

This difficulty in isolating the clotting factors also would seem to underlie a number of other divergent theories which have been evolved to explain the clotting process. These various theories have been discussed in several reviews of blood clotting in the last decade (262, 137, 93, 183) and will not be considered here.

Conversion of prothrombin into thrombin

1 *Nature of the reaction* The exact mechanism of the first, or conversion, stage of clotting remains one of the most important unsolved problems in blood coagulation. Many of the clotting theo-

ries differ mainly in their explanation of this reaction. Undoubtedly, the greatest obstacle to the complete elucidation of this reaction is our inability to obtain prothrombin and thromboplastin in pure form. The reaction may be influenced greatly by impurities in the preparations, particularly clotting inhibitors, which may even mask the true character of the reaction.

In general, two types of reaction have been considered in the conversion of prothrombin to thrombin: (a) an enzymic reaction, in which the conversion is catalyzed either by thromboplastin alone or by thromboplastin in conjunction with calcium, and (b) chemical combination of prothrombin with either thromboplastin or calcium or with both.

(a) *Enzymic reaction*. Morawitz originally termed the thromboplastic factor "thrombokinas," because he considered it an enzyme. Little experimental support for this thesis has been forthcoming, however, and the data available are mainly indirect. Particularly important in this connection are the recent studies on the clotting activity of trypsin. Older experiments had indicated that oxalated blood is clotted by trypsin (132). Similarly, Eagle and Harris (94, 95) found that trypsin in optimal amounts caused clotting of citrated plasma. From their studies with purified prothrombin (169) and crystalline trypsin, they concluded that, in a mixture of these two preparations, conversion to thrombin occurred, even in the absence of calcium. Thus, trypsin apparently substituted for both thromboplastin and calcium in clotting. They state that "It is difficult to conceive of so specific a phenomenon as the transformation of prothrombin to thrombin being effected by two wholly dissimilar mechanisms. We therefore suggest as a tentative working hypothesis that Ca plus platelets together constitute a proteolytic enzyme analogous to trypsin, which reacts with prothrombin to form thrombin." Eagle (92) found that the clotting action of certain snake venoms rich in proteolytic enzymes was due likewise to direct conversion of prothrombin to thrombin. In these experiments, no direct study of the proteolytic activity of thromboplastin was carried out.

Mellanby (172) also concluded that the coagulation of decalcified plasma by trypsin occurred through the intervention of thrombin. In his experiments he used commercial trypsin preparations and he

believed that clotting resulted from the calcium contained in the trypsin. In more recent studies, Mellanby and Pratt (173) used activated fresh pancreatic juice and stable fowl plasma, i.e., plasma to which no anticoagulant had been added. Although the pancreatic juice coagulated the fowl plasma, no coagulation occurred if the calcium was first removed by oxalation. In these experiments they interpreted the clotting action of trypsin as being due to liberation of thromboplastin from an inactive form normally present in fowl plasma. Ferguson and Erickson (103) have re-examined the question of trypsin clotting. From their data they believe that trypsin by proteolysis acts to make not only thromboplastin but also calcium available for clotting. In this case, trypsin clotting would have no bearing on the question of the enzymic nature of the prothrombin conversion reaction *per se*.

For many years the possibility had been considered that the interaction of prothrombin plus calcium, without thromboplastin and in the absence of inhibitors, would result in thrombin formation, as suggested by Howell (137). Recently Mellanby (170) found that his potent and highly purified prothrombin preparation was converted slowly into thrombin with calcium alone. With thromboplastin the conversion was rapid. Thus, it appeared possible that thromboplastin may merely catalyze the reaction, $\text{prothrombin} + \text{Ca} \rightarrow \text{thrombin}$. However, later investigations have indicated that Mellanby's prothrombin, although very potent, contained sufficient thromboplastin to account for the thrombin formation with calcium alone. Eagle (92) prepared Mellanby's prothrombin from plasma free of platelets, and hence free of the major source of thromboplastin. The product was not converted to thrombin in the presence of calcium alone. Mertz, Seegers and Smith (175) likewise have prepared a potent prothrombin which was not converted to thrombin in the presence of optimal amounts of calcium.

A number of investigators (93, 113, 123) have indicated that thrombin will catalyze the reaction, $\text{prothrombin} \rightarrow \text{thrombin}$. Such autocatalytic activity would point to the enzymic nature of this reaction. However, Astrup (35) recently has stated that the reaction is not autocatalytic. In a simple qualitative experiment, using prothrombin solution instead of plasma, he found that addition of pre-

formed thrombin did not influence the formation of thrombin from prothrombin

(b) Chemical combination In view of the data cited above, it appears unlikely that prothrombin and calcium react in the absence of thromboplastin to form thrombin (see also Ferguson (98))

Ferguson (99, 100) has suggested that all three of these clotting factors interact in the formation of thrombin It has been shown repeatedly that the amount of thrombin formed is directly proportional to the amount of prothrombin present, provided adequate calcium and thromboplastin are available for the reaction (90, 175) Ferguson's recent experiments suggest, in addition, that the quantities of both thromboplastin and calcium available are determining factors in the amount of thrombin formed He infers that in the reaction a prothrombin-calcium-thromboplastin "compound" or colloidal complex is formed, at least as an intermediate step in thrombin formation

Fischer believes that in the conversion reaction there is first a non-specific denaturation of prothrombin by thromboplastin, followed by the union of the denatured prothrombin protein with thromboplastin Fischer (112) has reviewed the experimental data on which this theory is based The role of calcium in this reaction is not defined

Recently Mertz, Seegers and Smith (175) have investigated the interrelationship of prothrombin and thromboplastin in thrombin production In contrast to earlier work, exact quantitative methods were employed in their experiments Using specially purified preparations of prothrombin and thromboplastin, with physiologically optimal amounts of calcium, they found that prothrombin and thromboplastin both are consumed in direct proportion to the amount of thrombin formed This would indicate a stoichiometric reaction involving prothrombin and thromboplastin The weight ratio of prothrombin to thromboplastin used up in the reaction is approximately 265 to 1 The preparations used were not completely pure, but even with a considerable increase in this ratio, it would still be relatively small in comparison to that of certain enzyme reactions in which the enzyme is "inactivated" in direct proportion to the amount of substrate transformed For example, it appears that under optimal conditions not more than one mol of catalase is "inactivated"

for each 300,000 mols of H_2O_2 decomposed into H_2O and O_2 (calculated from Morgulis' data (180))

Thus, much progress has been made recently in the elucidation of the prothrombin conversion reaction. The possibility that thromboplastin is an enzyme, at least of the traditional type, would appear to be eliminated. The evidence that both prothrombin and thromboplastin are consumed in the reaction strongly points to a direct chemical interaction. At present, the role of calcium would appear to be least clearly understood.

2 Factors influencing prothrombin conversion The rate at which prothrombin is converted to thrombin is known to depend upon the concentrations of thromboplastin and of prothrombin. In normal plasma, the slow coagulation time is due to the small amount of thromboplastin available. By greatly increasing the thromboplastin, the clotting time can be reduced to a minimum of a few seconds (see pp 341-343). Also, with a constant large amount of thromboplastin, the rate of thrombin formation is affected greatly by the prothrombin concentration (254). Calcium likewise is important, both small as well as excessive amounts decrease the speed of the reaction greatly. In addition, Ferguson (101) has suggested that the total quantity of thrombin formed from a *fixed* amount of prothrombin may vary, depending on the concentration of calcium, cephalin and prothrombin in solution—the greater the dilution of prothrombin, the less the total amount of thrombin formed from a fixed quantity of prothrombin. He suggested that only if this "dilution factor" is controlled, i.e., only if the concentration of prothrombin in the first phase of clotting is kept constant, will there be a constant relationship between the amount of prothrombin and the amount of thrombin which can be formed.

Electrolyte concentration, pH and temperature likewise are known to influence the prothrombin conversion reaction. Very little exact data are available concerning their effects, however, probably because a study of the influence of these factors on the isolated first stage of clotting involves a number of difficult technical problems. Many workers recently have been aware of the importance of these factors, and have kept them constant in their experiments. Unless this is done, caution must be exercised in interpreting experimental results.

Mellanby (169) has studied the influence of pH and temperature on the conversion rate of his purified prothrombin preparation. A pH of 7 was found to be optimal for the formation of thrombin, although over a wide range on the alkaline side (to pH 9) thrombin formed readily. At pH 6 the reaction was blocked completely. In his experiments thrombin formed with equal rapidity at 35° and 45°, but at lower temperatures (25° and 15°), the reaction rate was much slower. (See also Ferguson (101)). At 55°, the reaction could not be studied satisfactorily because of the thermolability of thrombin.

There appear to be a number of additional factors which may affect the speed of the prothrombin conversion reaction. One of these is the specific "convertibility" of the prothrombin. Warner, Brinkhous and Smith (253) have studied species differences in the convertibility of prothrombin. With amounts of calcium and thromboplastin which gave the shortest clotting time, they found that the conversion of prothrombin to thrombin in human or guinea pig plasma required two to four times as long as it did in dog or rabbit plasma. It was suggested that this slow prothrombin conversion is important in making man so susceptible to hemorrhagic diseases. Whether the variations in conversion speed are due to species differences in the prothrombin molecule or to unrecognized factors which influence the first phase of clotting is unknown. Owen, Hoffman, Ziffren and Smith (189) have observed patients in whom a decrease in the plasma prothrombin was compensated for apparently by an increased convertibility of the prothrombin. Schönheyder (216) has considered the possibility of species differences in plasma prothrombin, but his data relating to this subject are by no means convincing, since other differences, as, for example, species differences in thromboplastin, were not considered.

Another factor which is believed to influence the utilization of prothrombin in clotting is an inhibitor, so-called antiprothrombin. Howell and Holt (138) believed from their studies with heparin that this substance acts directly as an antiprothrombin. Many subsequent investigators obtained evidence which supported this finding. On the other hand, Mellanby (171), using his purified prothrombin preparation, showed that heparin did not influence the conversion of prothrombin to thrombin by calcium and thromboplastin. Quick

(196) obtained similar results on addition of calcium to prothrombin prepared by carbon dioxide precipitation. These findings suggested that the anticoagulant action of heparin was due only to the inhibition of the second or thrombin phase of clotting. This would imply that, in blood made incoagulable by heparin, the prothrombin is converted to thrombin, but that the thrombin is inactivated before it clots fibrinogen. Yet Warner, Brinkhous and Smith (251) found that heparinized plasma contained a normal amount of prothrombin. Further, this normal prothrombin value persisted for hours (48), a finding which definitely indicated that prothrombin conversion had been blocked by heparin. These divergent results concerning the influence of heparin on the prothrombin conversion reaction were explained by the recent finding of Brinkhous, Smith, Warner and Seegers (48) that a factor present in plasma or serum is required in addition to heparin for the antiprothrombic activity. Astrup (36) has confirmed this observation. This inhibitory activity probably should not be termed antiprothrombic, it could equally well be an antithromboplastic activity (102, 171).

It appears unlikely that this particular inhibition of thrombin formation is the cause of the slow conversion of prothrombin in human and guinea pig plasmas, as an excess of thromboplastin ordinarily overcomes the inhibitory effect of small amounts of heparin. The importance of the inhibitory action of heparin-plasma factor in normal clotting has not been determined, however.

Quantitative determination of prothrombin

Prothrombin has not been isolated in pure form, and relatively little is known of its chemical composition. The only means of recognizing this clotting factor is by its capacity to form thrombin, hence any method for the quantitative determination of prothrombin must be an assay based upon thrombin formation.

1 *Two-stage method for prothrombin determination (Warner, Brinkhous and Smith)* This method (221, 250, 251), first outlined in 1934, utilizes the biphasic nature of the clotting reaction and hence consists of two stages. In the first or prothrombin conversion stage, the prothrombin is converted completely to thrombin with an optimal amount of calcium and an excess of thromboplastin. In the second,

or clotting stage, the amount of thrombin formed is measured by the time required for the clotting of a standard fibrinogen solution

Notwithstanding the fact that the exact reaction in the first stage of clotting is not understood completely, it is well established that the amount of thrombin formed under standard conditions is directly proportional to the quantity of prothrombin present. Hence, the amount of thrombin formed is an exact index of the quantity of prothrombin originally present. In the second stage, the clotting time varies with the amount of thrombin, the greater the thrombin concentration, the shorter the clotting time. One unit of thrombin has been defined as the quantity which will under standard conditions cause clotting of 1 cc of a fibrinogen solution in 15 seconds. One unit of prothrombin is the amount required to form one unit of thrombin. Normal human plasma contains approximately 300 units of prothrombin per cc.

In the performance of this assay, serial dilutions of plasma are made before the prothrombin is converted to thrombin in the first stage. That dilution which will give a final concentration of one unit of prothrombin per cc is determined, and the dilution under these circumstances becomes an exact measure of the prothrombin unitage of the plasma. Human plasma, then, must be diluted 300 times before it is of unitary concentration.

The prothrombin concentration of plasma may also be expressed in per cent of normal by comparing the units of the unknown with those of a normal plasma. For example, if a patient's plasma contained 150 units of prothrombin per cc, the prothrombin concentration would be 50 per cent of normal.

Plasma, as well as other prothrombin-containing solutions, can be assayed readily by the two stage method. However, if plasma is being assayed, it must first be defibrinated with thrombin. Otherwise the fibrin clot, which would form during the first stage of the test, would interfere with the measurement of thrombin in the second stage of the test.

In this assay procedure all of the physico-chemical factors known to affect the prothrombin conversion reaction are controlled. Calcium, thromboplastin, total electrolyte and colloid concentrations as well as pH and temperature are kept constant. Also, complete

conversion of prothrombin to thrombin is assured in the first stage of the test

If the prothrombin of plasma is being assayed, it is possible that the antithrombin normally present in plasma will inactivate part of the thrombin before it can be measured in the second stage of the titration procedure. However, on dilution of plasma, its antithrombic activity becomes negligible, and this factor appears to be of no practical importance in the test. Even in human plasma in which the prothrombin is reduced to one-fifth of the normal value, the plasma is still diluted 60–70 times in this titration procedure. If the antithrombin of plasma is abnormally high, as in strongly heparinized plasma, the plasma globulin fraction, which contains practically all of the prothrombin and very little antithrombin, may be used instead of whole plasma (251).

Stewart and Rourke (231, 232) have used a modification of the method of Warner, Brinkhous and Smith for determination of prothrombin. The plasmas, both unknown and control, are diluted a fixed amount regardless of their prothrombin content. Also, the incubation period of the first stage is kept at a short, constant interval which is less than the time required for the complete conversion of prothrombin to thrombin. They have constructed a series of curves correlating prothrombin concentration with the clotting time in the second stage. Then, using standard conditions, the prothrombin concentration can be determined directly from the clotting time. This procedure has the theoretical disadvantage of not separating sharply the clotting process into its two phases, and any variations in convertibility of prothrombin would probably interfere with the accuracy of the test. However, in their work with prothrombin deficiency in patients, they apparently have obtained reliable results by this procedure.

2 *One-stage methods for prothrombin determination* Two methods have been developed. They differ chiefly in that one requires oxalated plasma for the test, the other whole blood.

a *Plasma prothrombin time (Quick)* This method (160, 191, 194, 197, 198, 203), first outlined in 1935, consists simply of determining the clotting time of oxalated plasma at 37.5°C, after addition of an excess of thromboplastin and a fixed amount of calcium. In a

plasma containing a normal amount of fibrinogen, the prothrombin concentration is the chief, although not the sole, variable affecting this accelerated clotting time. This prothrombin time should be distinguished from Howell's prothrombin time (135), in which the quantity of thromboplastin was not controlled.

With a given thromboplastin preparation, the normal plasma prothrombin time was found to be practically constant for each of the species tested. Depending on the activity of the thromboplastin used, the prothrombin time of normal human plasma varies between 10 and 25 seconds. If plasma is diluted to give prothrombin concentrations between 5 and 100 per cent of normal, a curve can be constructed to express the relationship between prothrombin time and prothrombin concentration. By use of such a curve, it is possible to interpret the results of this test in per cent of the normal prothrombin concentration. Recently Quick (200) has described an empirical equation which may be used as a substitute for the curve.

The prothrombin time for plasmas containing as little as 60 per cent prothrombin is nearly the same as for normal plasma. For this reason, a normal prothrombin time cannot be interpreted to mean that the plasma contains 100 per cent prothrombin. However, if prothrombin times are done not only on the whole plasma but also on the plasma after diluting two and even three times (159, 189, 202), a diminution in the prothrombin level can be determined more accurately.

This method for determination of prothrombin measures an accelerated clotting time which is made up, in part, of the prothrombin conversion time and, in part, of the time required for the thrombin formed to react with the fibrinogen. These two phases overlap, so that at the time of clotting, a large fraction of prothrombin has not yet been converted to thrombin (254). Thus, the use of an accelerated clotting time as an index of prothrombin concentration cannot depend on complete prothrombin conversion. Instead, it depends upon the assumption that an exact ratio exists between the rate of thrombin formation and the prothrombin concentration. However, it has been shown that the prothrombin conversion rate is variable, and is not dependent solely upon the amount of prothrombin present (see p. 337).

Stewart and Pohle (235) have investigated the recalcification procedure in the determination of the plasma prothrombin time. Instead of adding a fixed amount of calcium in the test, they varied the calcium over a wide range. They observed that the shortest prothrombin time was obtained if only 5–40 per cent of the usual amount of calcium were added. Quick (199) obtained similar results. Pohle and Stewart (190) found that the addition of an optimal amount of calcium gave a final concentration of plasma calcium of 8–13 mgm per cent. They have advocated, as a routine, the performance of a series of prothrombin time tests with each plasma, using varying amounts of calcium. Quick (199) believes that this modification is unnecessary, however.

In all of the experiments cited above with varying calcium concentrations, the total electrolyte concentration was not controlled, and with decreasing amounts of calcium, the tonicity of the clotting mixtures was decreased also. It is known that hypotonic mixtures clot more rapidly than isotonic ones, and the differences in clotting times obtained in these experiments may not have been due to calcium variations alone. It does appear, nevertheless, that variations in calcium are important, and that variations do occur in the prothrombin test of Quick. In anemic blood, for example, the plasma hematocrit is high, and the oxalate content is correspondingly low. It would appear that the modification by Pohle and Stewart would be helpful in controlling the calcium factor, but at the same time it would seem desirable that the tonicity be kept constant.

Several factors aside from actual prothrombin concentration affect the plasma prothrombin time. Variations in the convertibility of prothrombin have been mentioned. Heparin added to plasma in small amounts (0.1–0.2 units per cc) interferes but little with this test, but in larger amounts the prothrombin time is greatly prolonged (2, 195). Also, bile salts in high concentrations (400 mgm per cent) were found to cause prolongation of the plasma prothrombin time. In lower concentrations comparable to that in the plasma of cases of obstructive jaundice, the bile salts did not influence the test (2). Pohle and Stewart (190) observed that the plasma prothrombin time of normal plasma is shorter if the plasma is markedly lipemic.

b. "*Bedside test*" for *prothrombin activity*. This test, described in 1939 by Ziffren, Owen, Hoffman and Smith (263), consists of mixing

freshly drawn whole blood with a fixed, large amount of thromboplastin and determining the clotting time. This test is a simplification of the plasma prothrombin time, since whole blood is used instead of plasma (cp 203). This eliminates the difficulties associated with recalcification, as mentioned above.

The results of this test are expressed in terms of clotting activity

$$\text{Clotting activity (per cent)} = \frac{\text{clotting time of normal control}}{\text{clotting time of patient}} \times 100$$

As a measure of the exact quantitative prothrombin level of the blood, this test has many of the objections of the plasma prothrombin time. Since the prothrombin time is affected by so many variables, the originators of the test did not advocate that it be used as an exact measure of the prothrombin level. Nevertheless, a comparison of the results with the quantitative prothrombin level as determined by the two-stage test showed that it is a useful practical clinical test, especially in biliary tract disease.

The S-value of Schönheyder (215) and the R-value of Dam and Glavind (73) have been used as an index of prothrombin activity. These values are based on a comparison of the amount of thromboplastin required to give a constant clotting time of plasma. These methods appear to involve more assumptions and the values obtained seem to be related less intimately to the plasma prothrombin level than in the case of the other one stage methods already described.

It is apparent from this discussion that the accurate measurement of prothrombin is both theoretically and technically complex. Of the methods available, it appears that the two-stage method is based on the fewest assumptions. It has a wide applicability for the determination of prothrombin, both in plasmas and in other prothrombin-containing solutions. Technically, it offers some difficulties in the preparation and storage of the biological reagents required for the test. The one stage methods have the advantage of simplicity, but they appear to be an index of prothrombin alone only under special circumstances.

Properties and purification of prothrombin

Plasma prothrombin is associated with the pseudoglobulin fraction of the plasma proteins. Like the pseudoglobulins, it is salted out of

plasma between $\frac{1}{3}$ and $\frac{1}{2}$ saturation with $(\text{NH}_4)_2\text{SO}_4$ (56, 177) At pH 5.3 the prothrombin is largely precipitated from diluted plasma (169, 217) Also, it is precipitated by mixing acetone with plasma (136) As tested with collodion and cellophane types of membranes, prothrombin is non-dialyzable (56, 169, 217) It is adsorbed onto a number of colloidal preparations including $\text{Ca}_3(\text{PO}_4)_2$ (41), $\text{Mg}(\text{OH})_2$ (120), $\text{Al}(\text{OH})_3$ (121, 193), and colloidal starch (119) Prothrombin is adsorbed also by passing plasma through a Seitz filter (154)

Plasma prothrombin in solution is thermolabile It is destroyed by heating plasma for $\frac{1}{2}$ hour at 56°C In plasma kept at room temperature, and to a less extent at 5°C , the prothrombin slowly becomes inactive In human plasma kept at 2° – 5°C , the prothrombin is maintained at a normal or nearly normal level during the first 5 days (153, 232) Storage of blood for a longer period leads to a progressive decline in prothrombin activity However, at the end of three weeks, 60 per cent of the prothrombin still remains if the temperature is kept at 2° – 4°C (153) As judged by the plasma prothrombin time, the prothrombin in stored blood disappears considerably more rapidly (37, 153, 206)

Rapid freezing at low temperatures does not injure prothrombin Plasmas frozen with liquid oxygen (-183°C) (148) or solid CO_2 (-78.5°C) (221) retain their prothrombin activity In fact, the prothrombin in frozen plasmas (-25° to -40°) is maintained at the original level for weeks Freezing thus offers a simple means of preservation of plasma prothrombin

Many of the older methods for the preparation of prothrombin have been reviewed by Fuchs (118) Most of these methods yield crude and weak preparations A simple procedure for obtaining an active, albeit crude prothrombin preparation is to bubble CO_2 through diluted plasma (1, 90) This precipitates prothrombin as well as inert globulin and fibrinogen The fibrinogen can be removed from the final solution by addition of a small amount of thrombin (1) or by heating momentarily at 56°C (90)

The prothrombin preparation described by Mellanby in 1930 (169) was far more active per unit weight than any previously described The prothrombin, along with other globulins and fibrinogen, is precipitated from diluted oxalated beef plasma at pH 5.3 The pro-

thrombin is then selectively dissolved from the globulin precipitate by brief treatment with lime water. The final precipitate, obtained by reprecipitation at pH 5.3 and dried with acetone, is a white powder which is apparently indefinitely stable. It is insoluble in water, but is readily soluble in weak Na_2CO_3 solutions (pH 7). The more active preparations of Mellanby can be calculated to contain approximately 75 units of prothrombin per mgm, i.e., 1 mgm, after complete conversion to thrombin, will clot 75 cc. standard fibrinogen solution in 15 seconds. The yield appears to be less than 10 per cent of the original plasma prothrombin. The difficulty in this method, as pointed out by Mellanby himself, lies in the removal of the prothrombin from the original globulin precipitate by lime water. Unless this step is carefully controlled, a variable, and many times, an impotent product is obtained.

A prothrombin preparation, which is considerably more active than Mellanby's product, was described in 1938 by Seegers, Smith, Warner and Brinkhous (217). In this method the prothrombin, after precipitation from diluted plasma at pH 5.3, is adsorbed on $\text{Mg}(\text{OH})_2$. Then the prothrombin is separated from the adsorbent by CO_2 , followed by dialysis against water to remove a number of insoluble impurities. The final product, which is water soluble, is obtained as a dry powder by evaporation of the water. Prothrombin prepared by this method contains 150 units of prothrombin per mgm. The yield is about 12–15 per cent. By a modification of the method of Seegers and coworkers, prothrombin has been obtained which contains approximately 500 units per mgm (Seegers, unpublished data).

Mellanby (169) has shown that solutions of his prothrombin preparation are less thermolabile than prothrombin in native plasma. Heating at 60°C for 5 minutes does not destroy the prothrombin, although it does alter it in some way so that it is converted more slowly to thrombin with calcium and thromboplastin. This change in convertibility is more pronounced after heating at 100°C , and in addition part of the prothrombin is destroyed at this temperature.

Prothrombin is inactivated by trypsin (94, 95), by certain proteolytic snake venoms (92), and by purified thrombin solutions (174, 175).

Although Fuchs and Falkenhausen (118, 121) believed that prothrombin and complement midpiece were the same substance, this has not been borne out by recent work (93, 216, 261).

Prothrombin content of plasma, species variations

Human plasma from healthy adults contains about 300 units of prothrombin per cc (253) The normal prothrombin level is remarkably constant, with but little variation from individual to individual (46, 198, 232, 253) No sex variations in plasma prothrombin levels have been observed In infancy the prothrombin level is subnormal, this will be considered below, along with hemorrhagic disease of the newborn

The same constancy in adult prothrombin levels has been found in all the species studied The relative amounts present in the plasmas of different species, as observed by Warner, Brinkhous and Smith (253), are listed below The prothrombin level in dog plasma is arbitrarily set at 100

Dog	100	Chicken	50
Rat	95	Turtle	42
Cat	91	Sea Bass	31
Rabbit	89	Stingray	27
Human	84	Dog fish	8
Guinea Pig	53		

If the prothrombin content of human plasma is determined by the one-stage prothrombin time method, it is found to contain only about one-fifth as much prothrombin as does the plasma of dog, cat or rabbit (194, 198, 203), instead of four-fifths, as indicated by the two-stage method This discrepancy may be due to the relatively slow rate of prothrombin conversion in human plasma (253, 254), a factor which cannot be evaluated by a one-stage method of prothrombin determination Another factor which makes interpretation of comparative prothrombin values difficult is the possibility of a species specificity in thromboplastin which might affect the rate of thrombin formation as well as the completeness of prothrombin conversion (179, 194, 253)

The prothrombin content of various plasmas, listed above, indicates that a gradual increase in prothrombin occurs from the lower to the higher vertebrates This increase is associated with the gradual evolution of the thrombin-fibrin clotting mechanism In certain lower forms of animals, fibrin clotting is rudimentary, or entirely absent, and hemostasis is accomplished largely by agglutination of the circulating blood cells (219) In the higher forms, the prothrombin content of the plasma is greater, and the better developed fibrin

clotting mechanism replaces in large part the agglutination process in hemostasis

In mammals, the prothrombin is present in large excess over minimal needs for an efficient clotting mechanism (130, 197, 251) In dogs, for example, the prothrombin may be reduced to as little as one-fifth of the normal level without the development of a hemorrhagic tendency or of a prolongation of the clotting time or of the bleeding time (130, 222) In man, this factor of safety is not so great, the plasma normally contains only somewhat more than twice as much prothrombin as is needed to prevent the development of a hemorrhagic tendency

Although serum has been used in the past as a source of prothrombin, both for *in vitro* and *in vivo* use, it contains only a small and variable amount of this clotting factor In shed blood, thromboplastin is liberated and the conversion of prothrombin to thrombin starts immediately At the time of clotting, Warner, Brinkhous and Smith (251) found that 8 per cent of the prothrombin was converted to thrombin After clotting, the prothrombin continues to be consumed, and at the end of 3 hours only 5 to 10 per cent of the original prothrombin remains in the serum as a rule (44, 251) Traces of prothrombin may still be present after the serum has aged for many hours or even days

Origin of prothrombin

A decade ago it was widely held that prothrombin is formed in the bone marrow, probably in the megakaryocytes, and that it is released into the plasma from the platelets The experimental work dealing with the prothrombin content of platelets and bone marrow was reviewed by Wöhlisch in 1929 (262) and by Howell in 1935 (137)

A few years ago the question of the prothrombin content of platelets was studied again by Eagle (90) In experiments with thoroughly washed platelets, he was unable to demonstrate prothrombin in them except in one instance, in which the trace of prothrombin found probably was due to incomplete washing Ferguson (98) more recently was able to find only extremely minute traces of prothrombin in washed platelets Ferguson questioned the significance of such small quantities of prothrombin and stated that a platelet origin of plasma prothrombin was incompatible with his experimental findings Fur-

ther, but indirect, support of this thesis is given by the finding that in cases of thrombocytopenic purpura and aplastic anemia with marked reduction in the number of circulating platelets, the plasma contained a normal amount of prothrombin (211, 232)

Recent work on the source of prothrombin has all pointed to the liver Nolf, in 1908, and before him, Ansiaux and Corin in 1894 suggested that prothrombin (i e , thrombogen of Nolf) was formed in the liver (see Nolf (183)) However, no quantitative method was available for the determination of prothrombin until a quarter of a century later, and this view largely was disregarded in favor of the platelet origin of prothrombin

Hepatic toxins, phosphorus, chloroform, and carbon tetrachloride, and hepatectomy have been used in the recent studies of the relationship between the liver and plasma prothrombin The liver toxins have been used extensively as a means of producing liver necroses With severe liver damage there commonly develops a hemorrhagic tendency due to a marked diminution both in plasma fibrinogen and in plasma prothrombin Although the low fibrinogen in this condition was recognized many years ago (89, 116, 256), it was not until recently that the importance of a low prothrombin in the production of this bleeding tendency was appreciated Warner, Brinkhous and Smith (221, 251) first observed that at the height of the liver damage, the prothrombin, as well as the fibrinogen, was markedly decreased With regeneration of the liver, the prothrombin (221), like the fibrinogen (116), returned to normal levels More recently, Quick (198) has reported similar results Smith, Warner and Brinkhous (221) found that in mild chronic chloroform intoxication, in which the liver damage is less marked, the plasma prothrombin still fell to a bleeding level, while the fibrinogen was maintained at a normal concentration Again, as the liver recovered, the prothrombin returned to normal At the time of spontaneous hemorrhage, the liver showed little or no hemorrhage or fibrin formation—findings which suggest that prothrombin consumption was not increased These experiments with experimental liver injury indicate that a normal or nearly normal function of the liver is essential for the maintenance of the plasma prothrombin level

Further support of the conclusion that the liver is concerned with

prothrombin formation is furnished by hepatectomy experiments Warner (249) found that, following partial hepatectomy in rats in which 60-75 per cent of the liver was removed, there resulted a profound fall in the plasma prothrombin and at times fatal hemorrhage. Loss of as much as 70 per cent of the prothrombin was observed 6 hours after operation. The liver regenerated completely in 10-21 days, during which period the prothrombin level returned to normal. Control operations not involving the liver showed clearly that excessive prothrombin consumption was not the cause of the decline.

Warren and Rhoads (255) found in completely hepatectomized dogs a reduction in the plasma prothrombin, as indicated by plasma prothrombin times. In these experiments, the decline in plasma prothrombin was marked 6 hours postoperatively. Andrus, Lord and Moore (29) have obtained similar results following hepatectomy in dogs. The immediate and profound fall in prothrombin following hepatectomy is shown in the data from one of their experiments.

<i>Hours following hepatectomy</i>	<i>Prothrombin per cent</i>
0	100
1	57
5½	38
7½	20
9	17
10	14

Dam, Glavind, Lewis and Tage-Hansen (76) excluded the liver from the circulation in geese, but they found no evidence of a bleeding tendency, even in an animal that survived 26 hours. Instead, they found an improvement in the clotting power of the blood as indicated by their R-value determinations. No data concerning prothrombin content of the plasma are given, however.

The data which have accumulated recently thus indicate that (1) platelets contain little or no prothrombin and that they are not concerned with the origin of plasma prothrombin, and that (2) experimental injury to or removal of the liver results in a profound decrease in plasma prothrombin, and that regeneration of the liver following injury or partial removal results in a return of the prothrombin to normal levels. From this it may be concluded that the liver is essential for the maintenance of a normal plasma prothrombin level.

VITAMIN K

Discovery of vitamin K and its relationship to prothrombin formation

In the five years from 1929 to 1933 a hemorrhagic chick disease was observed, apparently independently, in a number of laboratories (63, 64, 134, 163, 164). In most cases the finding was an incidental one, noted in the course of experiments in which chicks were fed purified diets low in fats. The first such observation, by Dam (63), occurred in the course of studies on cholesterol metabolism. In 1931, while studying the effect of various proteins on chick growth, McFarlane, Graham and Hall (163) similarly noted spontaneous hemorrhages in their chicks. Similar findings were noted by McFarlane, Graham and Richardson (164) in their work on the fat-soluble vitamin requirements of the chick. It was considered originally that the hemorrhagic syndrome was due to scurvy, despite earlier reports that chicks are able to synthesize vitamin C. Cabbage, known to be rich in vitamin C, cured the hemorrhagic tendency (134). However, in later studies, scurvy as well as other known vitamin deficiencies were ruled out. Vitamin supplements, consisting of large amounts of lemon juice (64), ascorbic acid by mouth or subcutaneously (65, 78), cod liver oil (65, 78, 134, 163, 164) and wheat germ oil (66, 67), all failed to prevent the development of hemorrhages in chicks fed the purified diets. Cook and Scott (60, 61) believed that the hemorrhagic symptoms were the result of intoxication by certain nitrogenous bases present in fish meals which were part of their purified diets. This finding could not be confirmed by other workers (7). It was first suggested by Dam in 1934 (65) that this was a new dietary deficiency disease and his suggestion (66) in 1935 that the deficient antihemorrhagic factor be called vitamin K (Koagulations-Vitamin) has been adopted generally.

The deficient diets which caused the hemorrhagic chick disease were low in fats. However, the first definite evidence of the fat-soluble nature of the vitamin is found in the early work of McFarlane, Graham and Richardson (164). They noted that the fish meal used in their basal diet prevented the development of hemorrhages unless it had been extracted by ether. Later Almquist and Stokstad (22) showed that fish meal and yeast, both of which form large parts of many of the hemorrhage-producing purified diets, contain traces of the vitamin

This has led to the ether extraction of these substances before their incorporation in the basal diets used for the production of the disease. The fat soluble nature of the vitamin was shown conclusively by Dam (66, 67) and Almquist and Stokstad (21, 22), who obtained concentrates of the vitamin from natural sources by ether extraction.

Following the recognition of the hemorrhagic chick disease as a nutritional disorder, the relationship of this new dietary factor to blood clotting soon was established. McFarlane, Graham and Richardson (164) and Schønheyder (214) both observed that chicks with the hemorrhagic disease have a prolonged clotting time. Schønheyder (215) first suggested that the hemorrhagic tendency is the result of a hypoprothrombinemia. Qualitative studies of a number of clotting factors revealed no abnormality, and, by exclusion, a low plasma prothrombin was suspected. Definite evidence pointing to a lowered prothrombin level was obtained by Dam, Schønheyder and Tage-Hansen (81) in 1936. Crude prothrombin preparations were obtained from normal chick plasma by acetone precipitation and by acetic acid precipitation. Added to a mixture of K-avitaminous chick plasma and thromboplastic muscle extract, the prothrombin preparations corrected the clotting defect. On the other hand, similar preparations from K-avitaminous chick plasma were without effect. Also, addition of a watery emulsion of a vitamin K concentrate to the plasma-thromboplastin mixtures failed to restore the clotting time to normal. From this they concluded that the clotting abnormality was due to a low plasma prothrombin level, and that vitamin K itself did not possess prothrombic activity.

A more exact study of the prothrombin in hemorrhagic chick disease was made by Quick (197). He followed the plasma prothrombin time in chicks fed a vitamin K-deficient diet. The progressive fall in prothrombin is shown in the following data selected from his experiment.

<i>Days on K-deficient diet</i>	<i>Prothrombin per cent of normal chicks</i>
1	100
4	40
8	22
11	19
15	18
18	10

A distinct hemorrhagic tendency appeared when the low prothrombin levels were reached. Both the low prothrombin and the hemorrhagic tendency were cured by feeding vitamin K (alfalfa meal).

The first definite evidence that mammals may develop a vitamin K deficiency was shown, not as in chicks, by exclusion of the vitamin from the diet, but in conditions in which the absorption of this fat-soluble vitamin is interfered with because of absence of bile in the intestine. In such conditions there develop a hypoprothrombinemia and eventually a bleeding tendency which are cured by administration of vitamin K. This was shown first in rats by Greaves and Schmidt (128) in October 1937, in man by Warner, Brinkhous and Smith (252) in January 1938, and in dogs by Smith, Warner, Brinkhous and Seegers (222) in June 1938. Other mammals have not been studied. The data connected with these and similar studies will be considered below.

Hemorrhagic chick disease

Much of our knowledge concerning vitamin K has resulted from a study of the deficiency in chicks. The cure or prevention of hemorrhagic chick disease is the basis of the methods for assay of this vitamin, without which the rapid advances in the chemistry of this vitamin would have been impossible. Also, much of the available data concerning the metabolism of this vitamin has come from a study of this chick disease.

Hemorrhagic chick disease is characterized by subcutaneous, intramuscular and internal hemorrhages, prolonged bleeding from minor abrasions and a delayed clotting time, all the result of a low plasma prothrombin. Ansbacher (31) has made an analysis of the sites of superficial hemorrhages in a large series of deficient chicks. The abdomen was the most frequent site (23.8 per cent). Other common locations in his series were legs, shoulders and wings. The hemorrhages were located either subcutaneously or intramuscularly. Both Dam and Schönheyder (78) and Almquist and Stokstad (22) have stated that trauma is important in determining the occurrence and site of hemorrhages.

From studies of the plasma prothrombin level in hemorrhagic chick disease, it appears that hemorrhages do not occur until the prothrombin has declined to approximately 10–15 per cent of normal (197,

244) In the development of the disease, the appearance of hemorrhages is preceded regularly by a prolongation of the clotting time (31). The studies of Tidrick, Joyce and Smith (244) indicate that the clotting time is delayed only if the prothrombin level has declined to less than 30-40 per cent of that of normal chicks. Thus, early in the course of the disease when the vitamin deficiency is less severe, the plasma prothrombin may be reduced considerably and the clotting time still be normal. It would appear that plasma prothrombin determinations are essential if any but the more severe grades of deficiency are to be detected.

In the production of this disease it is essential that the diet be thoroughly freed of vitamin K. The presence of very small amounts of the vitamin will prevent the development of a severe deficiency. Satisfactory diets have been devised by Dam and Schönheyder (78), by Dam (67), and by Almquist and Stokstad (22). Almquist and Stokstad showed, as indicated previously, that both fish meal and yeast, which comprise a large part of many basal diets, contain traces of the antihemorrhagic factor. This led to the ether extraction of these substances before their incorporation in the diet. Although Dam and Schönheyder (79) found that the vitamin is extracted more completely from foods with acetone or 99 per cent alcohol than with ether, the latter has been used most commonly in the preparation of K-deficient diets. The diet finally used by Almquist and Stokstad (23) and widely adopted by other investigators consists of

Ether extracted fish meal	17.5 parts
Ether extracted dried brewer's yeast	7.5 parts
NaCl, with small amounts of CuSO_4 and FeSO_4	1.0 part
Cod liver oil	1.0 part
Ground polished rice	73.0 parts

This diet must contain very little vitamin K, since Almquist (7) has noted the hemorrhagic disease in chicks on this diet as early as the fifth day after hatching. Nevertheless, a certain number of chicks failed to develop hemorrhages or prolonged clotting times during a four-week period on the diet (19). Ansbacher (31) carefully reexamined the diet for traces of vitamin K. He observed that if the fish meal of the diet were extracted continuously with ether for seven days, a severe deficiency disease resulted more uniformly. In a group of 4321

chicks, over 95 per cent developed hemorrhages after being on the diet for 15 days or less

The vitamin is synthesized by bacteria, and hence bacterial growth in the basal diet must be prevented if the diet is to remain K-free. This apparently was observed first by Halbrook (quoted by Almquist and Stokstad (22)). It was found that if fish meal or rice bran were moistened before incorporation in the basal diet, the disease did not develop. This was true even if the fish meal were extracted with ether before being moistened. Evidently the bacterial growth produced enough vitamin K to prevent the development of the disease (22). Almquist, Pentler and Mecchi (20) isolated from putrefying fish meal a strain of bacteria which on growth developed abundant amounts of the vitamin. In fact, putrefying fish meal was used subsequently by Osterberg (188) and MacCorquodale, McKee, Thayer and collaborators (155, 165, 242) as a potent source of vitamin K.

An outgrowth of these same observations on the production of the vitamin in putrefying fish meal was the discovery by Almquist and Stokstad (23) that the vitamin is present in the feces, even if the chicks are kept on a vitamin K-free diet for a long period of time. Since feces of chicks having the disease contain vitamin K, the production of the vitamin must be in the lower part of the intestine, too low for absorption. Because of the vitamin K content of the feces, coprophagy must be prevented if the disease is to be produced successfully.

Even if carefully prepared basal diets are used and coprophagy is prevented, the severity of the disease and the age of onset of hemorrhages still appear to depend on certain other factors. The possibility of changes in vitamin K requirements with varying growth rates has been suggested by Almquist and Stokstad (22). In the early experiments with vitamin K deficiency, it was noted that the more rapid the growth, the more severe the disease (22, 134). Also, after the age of two weeks, chicks with hemorrhagic symptoms occasionally survived and showed no further hemorrhagic symptoms, even though they were continued on the K-deficient diet (22, 25). In a large group of chicks, Ansbacher (31) observed no "cures" of this type, however. No experiments have been reported in which the vitamin intake and growth rates were correlated with plasma prothrombin levels.

Another factor, also suggested by Almquist and Stokstad (23),

which influences the development of the disease is the difference in vitamin K reserves in newly-hatched chicks. If hens were fed on diets containing large amounts of alfalfa meal, rich in vitamin K, their eggs gave chicks which were more resistant to the development of the disease than normal controls. The transmission of the vitamin is apparently through the yolk, since yolk supplements to the basal diet protected against development of the chick disease, while egg albumen supplements did not. These stores in chicks on deficient diets are apparently exhausted at the end of two weeks. Seasonal variations in the vitamin reserves of chicks probably occur, as green plants are the most potent food sources of the vitamin. This would explain the observations of Thayer and associates (242) that the chick deficiency disease is most severe in the late winter and early spring months, and of Tidrick, Joyce and Smith (244) that normal chicks have a higher prothrombin level in the summer than in the late fall.

Even if the factor of varying reserves is controlled, individual variations in the susceptibility of chicks to the deficiency have been reported (19). The nature of these variations has not been determined. The possibility of refection, as in certain vitamin B experiments, has been pointed out (77). Although bile acids are important in the absorption of the vitamin, their administration failed to erase these individual differences (19). In this work, prothrombin determinations were not made. It is possible that if this were done, many of the seeming differences in susceptibility to the deficiency disease might disappear.

Since so many factors influence the production of the disease, it is not surprising that a severe grade of deficiency was not produced uniformly in the earlier stages of the work on vitamin K, and that some workers were unable to produce the disease at all (62).

In chicks with vitamin K deficiency, a number of other abnormalities aside from the clotting disturbance have been observed. Gizzard erosions (22, 78, 134) and anemia (60, 63, 78) were noted early in the study of hemorrhagic chick disease. There was a question for some time whether or not the gizzard changes were an integral part of vitamin K deficiency. Although it was observed repeatedly that many chicks with hemorrhage did not have gizzard erosions, and vice

versa, Dam, Schønheyder and Lewis (80) considered that erosions were as good a criterion for the identification of the disease as the presence of hemorrhages. Almquist (7), however, considered the gizzard lesions as separate from the disease, and in 1938 (10) he demonstrated that large doses of vitamin K did not protect against their development, although the clotting defect was prevented completely.

Divergent results have been obtained as to the basis of the anemia in hemorrhagic chick disease. It is not completely clear whether the anemia is secondary to the hemorrhages, or whether it is due directly to the vitamin K deficiency. Thayer and collaborators (243) found low red cell counts and low hemoglobin levels in deficient chicks. Although hemorrhages were not ruled out as the cause of the anemia, they observed complete return of both the erythrocyte count and the hemoglobin to normal levels after 3 days treatment with a potent vitamin K concentrate. Almquist (7) and Almquist, Mecchi and Klose (19) state that they have observed repeatedly a normal hemoglobin level in chicks with a greatly prolonged clotting time but without hemorrhages.

Dam and Glavind (73) observed that some of their chicks on a vitamin K-deficient diet developed encephalomalacia similar to the dietary encephalomalacia described by Goettsch and Pappenheimer (122). However, it would appear that these central nervous system lesions are not due to the vitamin K deficiency. Goettsch and Pappenheimer (122) and Mason (161) found that feeding of alfalfa to chicks on the encephalomalacia-producing diet failed to prevent the development of the encephalomalacia.

The response of deficient chicks to vitamin K has been studied by many workers. Dam, Glavind, Lewis and Tage-Hansen (76) studied the relative effectiveness of various routes of administration of a potent vitamin concentrate of alfalfa. Intravenous injection was found to be more effective than subcutaneous or intramuscular injections. By the intravenous route, the vitamin concentrate corrected the clotting defect at the earliest $3\frac{1}{2}$ hours after injection. By the intramuscular route, daily injections for 2-3 days were required as a rule. Subcutaneous injection of an emulsion of the vitamin concentrate was without effect, but by using a vitamin K concentrate previously heated with deoxycholic acid and in water solution, a response

was obtained in one day Almquist and Klose (13) studied the utilization of one of the pure compounds having vitamin K activity—phthiocol—and found it to be effective whether given intravenously, intramuscularly or by mouth

Ansbacher (30) has studied the rate of response following introduction of the vitamin directly into the crop With a minimal effective dose, the clotting time of a deficient chick was reduced from over 30 minutes to 6 minutes or less in 6 hours If a very large dose were given, a response was noted in as few as 2½ hours Tidrick, Joyce and Smith (244) also studied the rate of response to vitamin K, using prothrombin levels of the plasma instead of clotting times to determine the response to the vitamin In 18 hours one microgram of the vitamin preparation (2-methyl-1,4-naphthoquinone) was sufficient to restore the clotting time of a deficient chick to normal, i e., to raise the prothrombin level to 30–40 per cent of normal Eight times this amount was required to bring the prothrombin level to normal, however If a shorter test period were used, still larger amounts of the vitamin were required to elicit the same prothrombin response.

Assay of vitamin K

The methods for assay of vitamin K are based on the prevention or cure of vitamin K deficiency in chicks The criteria used for the detection of the disease have varied with different investigators and with the same investigator from time to time Originally the presence of hemorrhages was accepted as the measure of deficiency Only the most severe deficiencies are detected by this overt symptom, however, and we now know that hemorrhages result only when the prothrombin level has fallen to about 10–15 per cent that of normal chicks (197, 244) Later on, a prolongation of the blood clotting time was used by many workers Although whole blood clotting time has been used widely as a measure of deficiency, yet a moderate deficiency may exist and the clotting time be normal It is prolonged only if the prothrombin level is below about 30 to 40 per cent that of the normal chick (244) Schönheyder (215) and later Dam and Glavind (73) used S-values and R-values respectively to indicate the extent of the deficiency These values depend upon the amount of thromboplastin required to give a constant clotting time of 3 minutes Undoubtedly

the values obtained are influenced by the prothrombin level, but as Dam and Glavind (74) have indicated, changes in other clotting factors influence the results. Almquist and Klose (14) recently adopted a prothrombin time measurement which is undoubtedly a great improvement over the clotting time tests. In view of the findings of Tidrick, Joyce and Smith (244), the use of the two-stage method for prothrombin determination would appear to give an even more accurate index of vitamin K deficiency.

The assay methods may be classed as preventive and curative. A preventive method has been employed extensively by Almquist and coworkers for several years. In the method first described by Almquist (4), the test substance was added to the basal diet in varying levels and that level determined which prevented the appearance of gross hemorrhages in four weeks. Almquist and Stokstad (25) used a similar procedure except that they used normal whole blood clotting time as the criterion of prevention rather than absence of hemorrhages. The test material was mixed with the basal diet and fed to newly-hatched chicks until the age of two weeks, when the clotting time determinations were made. Deficient control chicks had a clotting time varying from 8 minutes to over 30 minutes. Their results are expressed as the level of test substance per kilo of diet which will prevent the development of the disease. Later Almquist, Mecchi and Klose (19) observed that more uniform results were obtained if all the chicks were fed on the basal diet alone for one week. Then supplements of the test substances were fed for the second week, at the end of which time whole blood clotting times were determined. Simultaneously other groups of chicks were fed supplements of a reference standard, that is, an extract of alfalfa meal rich in vitamin K. Then the amount of vitamin K in the unknown test substance was expressed in terms of the alfalfa reference standard. This modified procedure is no longer a strictly preventive method, since the chicks undoubtedly develop a moderate deficiency during the first week when they are fed only the basal diet. This assay procedure was revised again in 1939 (14) when a modified prothrombin time on whole oxalated blood was used instead of whole blood clotting time. In individual chicks the prothrombin time was found to be a more reliable index of the deficiency than the whole blood clotting time.

Almquist and Klose (14) observed that the reciprocal of the prothrombin time was directly proportional to the log of the vitamin K concentrate given per kilo of diet. From this empirical relationship, they determined from a very few feeding tests the vitamin K activity of an unknown material in terms of the standard alfalfa preparation.

Many of the pure vitamin preparations are inactivated readily by exposure to light and by other means. Hence, in these assay procedures in which the test material is mixed with the basal diet, there is the possibility that the vitamin will in part be inactivated before feeding, with a corresponding error in the assay value (15, 16, 17).

A large number of curative procedures for the assay of vitamin K have been devised. The first curative method was described by Schönheyder in 1936 (215). In this procedure, 35 day old chicks, on the deficient diet for 3 weeks, are given the test substance daily in tablet form for three successive days. The deficiency and its cure is recognized by determination of the so-called S- or "sickness" value, which is based on the relative amounts of thromboplastin required to give a 3 minute clotting time with carefully collected stable chick plasma. The Schönheyder unit of vitamin K is defined as the smallest daily dose per gram of chick which will correct the clotting defect in 3 days. Dam and Glavind (73) modified Schönheyder's original procedure in certain respects. They express the clotting capacity of the blood as an R-value $R = \frac{K}{K_n}$, where K equals the concentration

of the thromboplastin required to give a 3 minute clotting time of the test animal's plasma diluted half with Ringer's solution, and where K_n is the thromboplastin concentration required to give the same clotting time for diluted normal chick plasma. For normal chicks, R equals 1, while for K-avitaminous chicks it may be 150 or greater. The Dam Glavind unit is defined as the minimum amount of vitamin fed per gram body weight of chick per day for 3 successive days which will reduce to normal an R-value of 75 or higher. A standard spinach preparation containing 500 vitamin K units per gram is fed as a reference standard. Both Schönheyder's method and the Dam and Glavind modification are very laborious, since, to obtain the stable plasma required for the clotting studies, arterial blood must be collected carefully from several chicks.

A second curative assay method for vitamin K is the one devised by Thayer, MacCorquodale, McKee and Doisy (241, 242). Groups of 10 or more deficient chicks, fed on the basal diet for approximately two weeks, are given the test material directly into the crop on 3 successive days, and on the fourth day the clotting time is determined. They have defined a unit of vitamin K as that amount which will reduce the clotting time to 10 minutes or less in 50 per cent of a group of deficient birds. To keep the unit constant, corrections are made for possible variations in the response of different groups of chicks to vitamin K. Each lot of chicks used is tested with a reference standard of vitamin K. This procedure was later modified (242) so that only a single dosage of test substance was given, and the clotting time determined 18 hours later. Comparative assays by the two methods reveal that with the 18-hour test the unitage of a given vitamin K preparation is only about 75 per cent of that determined by the 3-day test. Using this 18-hour assay method, Binkley and associates (40) found that as few as two to four chicks could be used at each dosage level for the rapid determination of the approximate vitamin K unitage of an unknown preparation.

The third curative assay method is that of Ansbacher (31). He made especially comprehensive studies to obtain chicks with a uniform vitamin K deficiency. The material to be tested for vitamin K is dissolved in a minimal volume of cod liver oil—larger amounts of the oil interfere with the activity of the vitamin—and is introduced directly into the crop of a deficient chick. Six hours later the clotting time is determined. Six minutes is used as a normal time. A unit of vitamin is defined "as the minimum amount necessary to render the blood clotting time of the vitamin K-deficient chick, weighing 70 to 100 gm., normal within 6 hours after administration." The test substance is fed in different dosage to several groups of five or more chicks to determine this minimum level. Recently Ansbacher, Fernholz and MacPhillamy (34) showed that certain of the more complex, pure vitamin K preparations require a longer time for utilization by the chicks. If an 18-hour instead of a 6-hour test period is used, a more accurate index of the vitamin K potency is obtained.

The fourth curative assay procedure for the determination of vitamin K is that of Dann (85). Groups of approximately 20 deficient chicks are given varying levels of the test material orally for 3 suc-

cessive days Then clotting times are determined At the same time another group of deficient chicks is treated with a standard vitamin K concentrate prepared from alfalfa This standard has been assigned arbitrarily a value of 5000 vitamin K units per gram, and by comparing the results obtained by feeding the unknown and the standard, the vitamin K unitage of the test material is determined

No adequate experimental data are available from which one can correlate exactly the various units of vitamin K Although several identical preparations have been assayed by two or more methods, numerous discrepancies are observed The following are comparative values which must be considered only as rough approximations These comparisons were made from the work of Dam (68), Ansbacher (31), Almquist and Klose (17), Thayer and coworkers (242), Karrer and Geiger (144) and Dann (86)

1 Ansbacher unit = 20 Dam Glavind units

1 Thayer-Doisy unit = 30 Dam Glavind units

1 Dann unit = 25 Dam Glavind units

100-150 Thayer-Doisy units per kilo of diet are protective by the method of Almquist, Mecchi and Klose (19)

With the discovery of pure naphthoquinone compounds possessing vitamin K activity, it was suggested by Thayer and coworkers (238) that one of the simplest and most potent of these, 2-methyl-1,4-naphthoquinone, be adopted as a standard in chick assay work, and all other materials be expressed in terms of the activity of 1 microgram of this compound This would simplify considerably the individual unit systems used at present by various investigators Almquist and Klose (17) used this procedure in their assay of vitamin K₁, 2 methyl-3 phytyl-1,4-naphthoquinone According to Thayer and associates (242), vitamin K₁ contains 1000 Thayer-Doisy units per milligram This is equivalent to approximately one-fourth milligram of 2 methyl-1,4-naphthoquinone (17, 104)

The possibility of a chemical means of determining vitamin K activity was suggested by the discovery of a color reaction for vitamin K by Dam, Karrer and coworkers (69) Vitamin K isolated from alfalfa gave on mixing with sodium alcoholate a characteristic color reaction The chemical nature of this color reaction has been studied

by Fieser, Campbell and Fry (111), Riegel, Schweitzer and Smith (207) and Almquist and Klose (18) Almquist and Klose (12) reported that the quantity of pigment formed in this color reaction is proportional to the vitamin K activity of the concentrate However, Fernholz, Ansbacher and Moore (105) could not confirm this They obtained in the course of purification studies on vitamin K an inactive material which gave a strong color reaction and an active material which gave a very feeble color reaction From these experiments, it appears that this chemical test will not give an accurate index of vitamin K activity

Chemistry of vitamin K

1 *Properties.* Early in the study of vitamin K it was observed that the antihemorrhagic factor is extractable from natural sources by fat solvents Early attempts at isolation of the vitamin showed that it was not in the sterol fraction of the lipids (22, 67) Lichtman and Chambers (150) have described a sterol preparation from liver with vitamin K activity, but this finding has not been confirmed

Saponification of the vitamin concentrates with alkali resulted in destruction of a large part of their activity, even in the cold (4, 79) In addition to being alkali-labile, the vitamin was found to be inactivated by light Exposure to sunlight destroyed the vitamin activity of alfalfa concentrates within 2 hours, although in artificial light (500 watt lamp) no destruction was observed in 24 hours (6) However, the activity of pure or nearly pure preparations of the vitamin from natural sources is destroyed both by artificial light and by sunlight in a few hours (155) The vitamin in concentrates of alfalfa or of hog liver fat resists heating up to 120° for 24 hours The vitamin is destroyed by oxidizing agents, strong acids, and AlCl_3 , and in reactions which eliminate ethylenic linkages (145) The vitamin is adsorbed on a number of materials, including calcium sulfate (68), weakly acidic fullers' earth (207), activated carbon, permutit, and decalco (synthetic zeolite) (40) Elution of the vitamin can be accomplished with certain solvents, such as petroleum ether Almquist (5) noted originally that crude preparations of the vitamin can be concentrated by molecular distillation

2 *Purification of vitamin K* Following the discovery in 1935 by

Almquist and Stokstad (22) that alfalfa meal is an excellent source of the vitamin, this material has been used extensively in the preparation of concentrates of the vitamin. By making use of certain properties of the vitamin in a series of purification procedures, mainly differential solubility, chromatographic adsorption and elution, and molecular distillation, in various combinations, successively more potent concentrates, as determined by chick assays, were prepared from alfalfa, until finally the vitamin was isolated in pure form. The first preparation from alfalfa, rich in vitamin K, was made by Almquist in 1936 (4). By distillation, a yellow oil, very rich in anti-hemorrhagic activity, was obtained (5). Concentrates by improved methods and usually with greater potency were obtained by Almquist (9), Dam and Lewis (77), Riegel, Schweitzer and Smith (207), and Dam, Karrer and coworkers (69).

Attempts were made to crystallize the vitamin from potent but impure concentrates by Almquist (8, 9) and by Thayer and collaborators (240). In 1937, Almquist (8, 9) obtained colorless crystals from solutions cooled to a low temperature with solid carbon dioxide. At higher temperatures, the crystalline material formed an oil, but the exact melting point was not determined. When added to the basal diet this oil prevented the development of hemorrhagic chick disease at a level of 2 mgm per kilogram of diet. This work has not been confirmed, and the crystalline material has not been compared to the known pure vitamin which, since then, has been isolated and synthesized. In 1938, Thayer and coworkers (240) reported the isolation from alfalfa of a crystalline preparation with a melting point of 69° and an extremely high vitamin activity. Later, however, they were unable to repeat this work (40, 155).

In attempts to purify further the vitamin concentrate, Almquist and Klose (11) reported the preparation of a vitamin K-choleic acid, which they believed was a molecular compound of the vitamin with deoxycholic acid. A crystalline material with a melting point of 186°-187°C was obtained. Chick assays indicated it contained about 10 per cent vitamin K. However, Riegel, Schweitzer and Smith (207) and Binkley and coworkers (40) were unable, in their experiments, to obtain such a compound with vitamin K activity.

Apparently the first practically pure vitamin K preparation isolated

from alfalfa was reported by Dam, Geiger, Karrer, Karrer, Rothschild and Salomon in January, 1939 (69). This was a clear yellow oil which contained 20 million Dam units per gram, or approximately 75,000 times more per unit weight than dried alfalfa. Thus this preparation was many times more active than the concentrates previously described. McKee (165), Binkley (39, 40) and collaborators obtained a similar highly potent oil from alfalfa, which possessed many of the properties of the oil reported by Dam, Karrer and co-workers. Binkley and associates (40) obtained the vitamin isolated from alfalfa in crystals in the form of yellow rosettes by cooling to low temperatures. The melting point was about -20°C . This preparation had a potency of approximately 1000 Thayer-Doisy units per mgm.

In the spring of 1939, it became apparent that more than one compound possessed vitamin K activity. MacCorquodale (155), McKee (165) and coworkers reported two distinctly different compounds with very high vitamin activity. These were isolated from alfalfa meal and from putrefying fish meal respectively. They called the vitamin from alfalfa vitamin K_1 , and the vitamin from fish meal vitamin K_2 . This terminology has been adopted widely, although Karrer and Geiger (144) have suggested another term for vitamin K_1 , α -phyloquinone.

In a surprisingly short time following the isolation of vitamin K_1 from alfalfa, its structure was identified and the compound was synthesized. During the earlier work with impure vitamin concentrates, certain chemical properties had been observed which indicated something of the composition of the vitamin. Almquist's data (6, 9) indicated that it was a complex cyclic unsaturated compound, mainly hydrocarbon in composition and free of nitrogen. The presence of ethylenic linkages was indicated by the work of Klose, Almquist and Mecchi (145). Although they were not dealing with a pure substance, the freezing point depression of camphor indicated a molecular weight of about 525 (145). Analyses of the oil isolated by Dam, Karrer and associates (69) showed it to be composed of only carbon, hydrogen and oxygen, with two atoms of oxygen per molecule. From the absorption spectra, the lability to light and alkali, the yellow color, and the hydrogenation reactions, it was suggested first by McKee

(165), Binkley (39) and associates and later, apparently independently, by Karrer and Geiger (144) that vitamin K_1 had a quinoid structure. That a quinoid structure was probably correct was indicated in June, 1939 by the report of Almquist and Klose (13) that a known quinone, 2-methyl 3-hydroxy-1,4-naphthoquinone (phthiocol), had vitamin K activity. That this compound as well as many others with the basic structure of 1,4-naphthoquinones have vitamin K activity was confirmed very soon by a number of workers (15, 32, 109, 110, 239).

For the final elucidation of the structure of vitamin K_1 , Doisy and associates (38, 156, 157, 158), Almquist and Klose (16, 18), and Fieser and associates (106, 107, 108, 109, 110, 111) are responsible. From a review of the literature on the chemical and physical properties of vitamin K_1 , i.e., adsorption spectra (39, 69), elementary analyses (69), hydrogen uptake (165), sensitivity to light and alkali, color, resistance of its diacetate to alkaline hydrolysis (39), Fieser and associates (109, 111) postulated that the structure was that of a 2,3-dialkyl 1,4-naphthoquinone. Hydrogenation experiments indicated that the side chain had one double bond. A naturally occurring alcohol, phytol, would satisfy this requirement, and with one or two methyl groups, the empirical formula would be satisfied. The structure then was suggested to be a 2,6(?)-dimethyl 3 phytyl-1,4-naphthoquinone or the 2 mono-methyl compound. From the study of less complex models, Fieser, Campbell and Fry (111) favored the latter structure. On the basis of a study of degradation products of vitamin K_1 , MacCorquodale and coworkers (156) simultaneously reached a very similar conclusion, differing only in the identity of the radical (ethyl) in the 2 position. Further study of degradation products by Binkley and collaborators (38) indicated that vitamin K_1 is 2 methyl 3 phytyl-1,4-naphthoquinone (see fig 1). The final step in the proof of the structure of vitamin K_1 was the synthesis of the compound in three separate laboratories practically simultaneously (Almquist and Klose (16), MacCorquodale and associates (157, 158), and Fieser (106, 107, 108)). The natural and synthetic products had the same physical and chemical properties and approximately the same antihemorrhagic activity. This comparison with the synthetic product showed conclusively that the structure of the

vitamin isolated from alfalfa was 2-methyl-3-phytyl-1,4-naphthoquinone

The data of Ansbacher, Fernholz and MacPhillamy (34) suggest that 2-methyl-3-phytyl-1,4-naphthoquinone may be only one of several vitamin K compounds present in alfalfa extracts. This is indicated by variations in the rate of utilization of synthetic vitamin K₁ and alfalfa concentrates as well as by their purification studies with chromatographic adsorption.

Vitamin K₂ was isolated from putrefying fish meal by MacCorquodale (155), McKee (165, 166) and collaborators by methods similar to those used for the isolation of vitamin K₁. This vitamin compound was obtained as light yellow crystalline plates with a melting point of 53.5°–54.5°C. The potency of vitamin K₂ is 60 per cent that of vitamin K₁, i.e., approximately 600 Thayer-Doisy units per mgm. Although vitamin K₂ has been isolated, its structure has not been identified. The data of McKee and coworkers (166) indicate that it also is a 2,3-disubstituted-1,4-naphthoquinone. Fieser and coworkers (109, 111), on the basis of the chemical and physical properties reported by McKee and associates (165), postulated the structure 2,3-difarnesyl-1,4-naphthoquinone (see fig. 1). Farnesol is a naturally-occurring unsaturated complex alcohol known to be present in certain fish oils in the form of di-farnesyl (squalene).

3 Synthetic compounds with vitamin K activity Almquist and Klose, in June 1939 (13) reported the first completely identified form of vitamin K. This compound is phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) (fig. 1) and might be termed vitamin K₃. In contrast to many vitamin K preparations, this compound is slightly soluble in water. A 0.2 per cent buffered solution was found to be effective in curing K-deficient chicks when given by mouth, intramuscularly or intravenously. Phthiocol was recognized originally by Anderson and Newman in 1933, several years before its vitamin K activity was suspected (27). It was isolated from the acetone fraction of tubercle bacilli. It forms yellow prismatic crystals which melt at 173°–174°C and which give deep red, water-soluble salts with bases. Synthesis has been accomplished by a number of methods (26, 28, 182).

Almquist, Pentler and Mecchi (20) demonstrated in 1938 that

cultures of tubercle bacilli possess vitamin K activity. Since phthiocol was isolated originally from tubercle bacilli, it appeared that phthiocol was a third naturally occurring vitamin K. However, Fieser, Campbell and Fry (111) have suggested that it may be an alkaline cleavage of vitamin K₁ or a homolog, since the isolation procedure used by Anderson and Newman (27) involved a saponification step.

Another synthetic naphthoquinone which has been studied extensively is 2-methyl-1,4-naphthoquinone (fig 1) or vitamin K₄. The vitamin K activity of this compound was reported simultaneously by four different groups of investigators (15, 32, 110, 239). In the original reports there was marked discrepancy between the various groups as to the vitamin K potency of this compound. Subsequent investigations (16, 17, 106, 238, 245) indicate that this compound is extremely potent and that it is even more active than vitamin K₁. This high activity was recognized by Ansbacher and Fernholz (32) in their original report. Subsequently they reported (104) that 0.5 microgram is equivalent to one Ansbacher unit. In comparison, they found that 2000 micrograms of phthiocol contain one Ansbacher unit of vitamin K. Like a number of other vitamin K compounds, 2-methyl-1,4-naphthoquinone is unstable unless special precautions are taken. It is very slightly soluble in water, and in solution, its vitamin K activity is impaired markedly by sterilization with steam at 15 pounds pressure for 30 minutes (114). Phthiocol, treated similarly, retains its activity.

Many of the synthetic compounds which have been tested for vitamin K activity, including vitamins K₁, K₃, and K₄, are listed in table 1. Most of the active compounds are basically 1,4-naphthoquinones or the corresponding hydroquinones. A few, however, are not. The simplest compound with vitamin K activity is 2,5-dimethyl-benzoquinone (phlorone) (33). Many of the 1,4-naphthoquinone compounds are inactive. Often relatively minor changes in the substituent groups of very active compounds of this type may completely eliminate their vitamin K activity. Thus, 2-methyl-1,4-naphthoquinone is very active, the 2-ethyl compound shows much less activity, and the 2-propyl compound is inactive (fig 2A). 2,3-Dimethyl-1,4-naphthoquinone is moderately active, but the isomer, 2,6-dimethyl-1,4-naphthoquinone is inactive (fig 2B). Also,

TABLE 1

Vitamin K Activity of Naphthoquinones and Certain Other Compounds

NUMBER	COMPOUND	VITAMIN K ACTIVITY	REPORTED BY	DATE
I Active compounds				
1	Phthlocol (2-methyl-3-hydroxy 1 4 naphthoquinone) (vitamin K ₂)	10 mgm per kg diet effective (19) 20 mgm per kg diet effective (14); equivalent to 270 gm standard alfalfa per gm 1 mgm. = 2 Thayer Dotsy units Less than 1 mgm = 1 Ansbacher unit 20 mgm. per kg diet effective (14) equivalent to 287 gm. standard alfalfa per gm. 2 mgm. = 1 Ansbacher unit	Almquist and Klose (13) Almquist and Klose (15) Thayer and associates (239) Ansbacher and Fernholz (32) Almquist and Klose (16) Fernholz and Ansbacher (104)	June 1939 July 1939 July 1939 July 1939 September 1939 September 1939
2	2 Methyl 1 4-naphthoquinone (vitamin K ₃)	1 mgm. = 15 Dann units 20 mgm per kg diet effective (14); equivalent to 435 gm standard alfalfa per gm 0.5 microgram = 1 Ansbacher unit 5 mgm daily dose for 3 days curative (Thayer method) 1 mgm = 10 Thayer Dotsy units 2.5 mgm per kg diet effective (14) equivalent to 5150 gm standard alfalfa per gm 1 microgram curative in 18 hours (modified Ansbacher method) 1 mgm = 1050 Thayer Dotsy units 1 microgram curative (modified Ansbacher method) 0.5 microgram = 1 Ansbacher unit 1 mgm equivalent to 240 gm standard alfalfa 1 mgm = 2500 Dann units 0.2 mgm. per chick curative in few hours 2-4 micrograms curative (modified Ansbacher procedure) 1 mgm. equivalent to 63 gm. standard alfalfa About 7.5 micrograms = 1 Ansbacher unit 1 mgm. = 750 Dann units	Dann (85) Almquist and Klose (15) Ansbacher and Fernholz (32) Kuhn and associates (149) Thayer and associates (239) Almquist and Klose (16) Fieser (106) Thayer and associates (238) Tishler and Sampson (245) Fernholz and Ansbacher (104) with MacPhillamy (34) Almquist and Klose (17) Dann (85) Almquist and Klose (16) Fieser (106) Almquist and Klose (18) Ansbacher Fernholz and MacPhillamy (34) Dann (85)	November 1939 July 1939 July 1939 July 1939 September 1939 September 1939 September 1939 September 1939 September 1939 November 1939 October 1939 November 1939 September 1939 September 1939 October 1939 November 1939 November 1939
3	2 Methyl-3-phytyl 1 4 naphthoquinone (vitamin K ₄)			

TABLE 1—*Continued*

NUMBER	COMPOUND	VITAMIN K ACTIVITY	REPORTED BY	DATE
I. Active compounds— <i>Continued</i>				
4	2 Methyl-3 "isophytol"-1,4-naphthoquinone	About 15 micrograms = 1 Ansbacher unit	Ansbacher, Fernholz and MacPhillamy (34)	November 1939
5	2,3 Dimethyl-1,4 naphthoquinone	250 micrograms very effective (modified Ansbacher method)	Fieser and co-workers (109)	July 1939
		500 micrograms daily for 3 days curative (Thayer method)	Kuhn and associates (149)	July 1939
		8 mgm per kg diet effective (19)	Fieser and associates (111)	August 1939
		50 micrograms curative (modified Ansbacher method)	Tishler and Sampson (245)	September 1939
6	2-Hydroxy-1,4-naphthoquinone	100 mgm per kg diet effective (14), equivalent to 84 gm standard alfalfa per gm	Almquist and Klose (15)	July 1939
		10 mgm daily dose for 3 days curative (Thayer method)	Kuhn and associates (149)	July 1939
		75 mgm per kg diet effective (14), equivalent to 139 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
7	2-Ethyl-1,4-naphthoquinone	1 mgm = 8 Thayer-Dolisy units	Thayer and associates (239)	July 1939
		Over 200 micrograms effective (modified Ansbacher method)	Tishler and Sampson (245)	September 1939
8	2-Methyl-3-cinnamyl-1,4-naphthoquinone	100 micrograms active (modified Ansbacher method)	Fieser (108)	November 1939
9	2-Methyl-3 geranyl-1,4-naphthoquinone	25 micrograms active (modified Ansbacher method)	Fieser (108)	December 1939
10	Phthiocol ethyl ether	15 mgm per kg diet effective (14), equivalent to 100 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
11	Phthiocol octadecyl ether	20 mgm per kg diet effective (14), equivalent to 95 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
12	Phthiocol phytol ether	20 mgm per kg diet effective (14), equivalent to 50 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
13	Phthiocol triacetate	15 mgm per kg diet effective (14), equivalent to 192 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
14	Phthiocol monoacetate	Less than 20 mgm per kg diet effective (14)	Almquist and Klose (15)	July 1939
		15 mgm per kg diet effective (14) equivalent to 420 gm standard alfalfa per gm	Almquist and Klose (16)	September 1939
15	2 Methyl 1 4 naphtho-hydroquinone	1 mgm = 1000 Thayer-Dolisy units	Thayer and associates (238)	September 1939
		1 mgm = <2000 Dann units	Dann (85)	November 1939
16	1,4-Naphthohydroquinone-diacetate	1 mgm = 0.5 Thayer-Dolisy units	Thayer and associates (239)	July 1939

TABLE 1—Continued

NUMBER	COMPOUND	VITAMIN K ACTIVITY	REPORTED BY	DATE
I. Active compounds—Continued				
17	2-Methyl 1 4-naphtho- hydroquinone-di acetate	About 5 micrograms = 1 Ans- bacher unit 1 mgm = 5 Thayer Doley units 2 micrograms = 1 Ansbacher unit About 1 microgram = 1 Ans- bacher unit	Ansbacher and Fernholz (32) Thayer and associates (239) Fernholz and Ansbacher (104) Ansbacher Fernholz and MacPhillamy (34)	July 1939 July 1939 September 1939 November 1939
18	2-Methyl-3-phytyl 1 4- naphthalenediol di acetate	1 mgm = 660 Thayer Doley units About 15 micrograms = 1 Ans- bacher unit	MacCorquodale and as- sociates (158) Ansbacher Fernholz and MacPhillamy (34)	September 1939 November 1939
19	2 Bromo-3-methyl 1 4- naphthoquinone	1 mgm. = >0.1 Thayer Doley units	Thayer and associates (239)	July 1939
20	2 3-Dibromo-2-methyl- 1 4-dibromotetrahydro- naphthalene	1 mgm = >0.1 Thayer Doley units	Thayer and associates (239)	July 1939
21	2 Allyl-4-amino-1-naph- thol hydrochloride	Active	Thayer and associates (239)	July 1939
22	4-Amino-2-methyl 1 naphthol	1 mgm contains about 1000 Thayer Doley units	Doley and associates (83)	November 1939
23	1 4-Dihydroxy 2-methyl 3-naphthaldehyde	5-10 mgm intravenously ef- fective in patients	Snell and Butt (279)	December 1939
24	2 Methyl-1 4-naphtho- quinhydrene	1 mgm = 2500 Dann units	Dann (25)	November 1939
25	α -Tocopherylquinone	10 mgm daily for 3 days cura- tive (Thayer method)	Kuhn and associates (149)	July 1939
26	2 5-Dimethylbenzo- quinone (phlorone)	1 mgm = about 1 Ansbacher unit	Ansbacher and Fernholz (33)	November 1939
II Inactive or slightly active compounds				
27	1 4 Naphthoquinone	1 mgm = 1 Thayer Doley unit 10 mgm daily for 3 days cura- tive (Thayer method) 100 mgm per kg diet inac- tive (14)	Thayer and associates (239) Kuhn and associates (149) Almqvist and Klose (16)	July 1939 July 1939 September 1939
28	2 7 Dimethyl 1 4 naph- thoquinone	200 micrograms active (modi- fied Ansbacher method) 400 micrograms inactive (modified Ansbacher assay) No appreciable activity	Fieser and associates (110) Tahler and Sampson (213) Fieser (108)	July 1939 September 1939 November 1939
29	Lomatol	250 micrograms effective (modified Ansbacher method) 100 mgm. per kg diet inac- tive (14) 10 mgm per kg diet inactive (19)	Fieser and co-workers (109) Almqvist and Klose (15) Fieser and associates (111)	July 1939 July 1939 August 1939

TABLE 1—*Concluded*

	COMPOUND	VITAMIN K ACTIVITY	REPORTED BY	DATE
II Inactive or slightly active compounds— <i>Continued</i>				
0	Hydroxyhydrolapachol	250 micrograms effective(modified Ansbacher method) No appreciable activity	Fieser and co-workers (109) Fieser (108)	July 1939 November 1939
1	2- α Heptenyl-3-hydroxy-1,4-naphthoquinone	200 micrograms, some activity (modified Ansbacher method) No appreciable activity	Fieser and associates (110) Fieser (108)	July 1939 November 1939
2	2-N-Heptyl 3 hydroxy-1,4-naphthoquinone	200 micrograms, some activity (modified Ansbacher method) No appreciable activity 2 mgm inactive	Fieser and associates (110) Fieser (108) Thayer and associates (239)	July 1939 November 1939 July 1939
3	2-Allyl-1,4-naphthoquinone	200 micrograms, very active (modified Ansbacher method) Over 200 micrograms, inactive (modified Ansbacher method)	Fieser and associates (110) Fieser and associates (111)	July 1939 August 1939

The compounds listed below are devoid of vitamin K activity, as indicated by all reported assays. The maximum amounts administered in these tests are indicated

- 1 2-Naphthoquinone (5 mgm) (239)
- 2,6-Dimethyl 1,4-naphthoquinone (0.4 mgm) (108, 110, 245)
- 2-N-Propylnaphthoquinone (0.4 mgm) (245)
- Lapachol (5 mgm , 100 mgm per kg diet) (15, 109, 111, 149)
- 2-Allyl-1,4-naphthoquinone (2 mgm) (239)
- Lomatol methyl ether, m p 61.5°-62° (0.25 mgm) (108, 109)
- Lapachol methyl ether (0.25 mgm) (109)
- 2-Methyl-3-benzyl-1,4-naphthoquinone (0.1 mgm) (108)
- 2-Methyl-3-trimethylallyl-1,4-naphthoquinone (0.2 mgm) (108)
- 2-Ethyl-3-phytyl 1,4-naphthoquinone (no data) (108)
- 2,6-Dimethyl-3-phytyl 1,4-naphthoquinone (0.05 mgm) (108)
- Hydrolapachol (0.25 mgm , 100 mgm per kg diet) (16, 108, 109)
- 2,3-Diallyl-1,4-naphthohydroquinone diacetate (0.2 mgm) (110)
- 1,4-Benzoquinone (5 mgm , 100 mgm per kg diet) (16, 33, 239)
- Toluquinone (5 mgm) (33, 239)
- p-Xyloquinone (5 mgm) (239)
- Trimethylquinone (1 mgm) (33)
- Duroquinone (1 mgm) (32)
- Thymoquinone (5 mgm) (239)
- Diallyl-1,4-benzoquinone, m p 16° (0.25 mgm) (109)
- Diallyl 1,4-hydroquinone, m p 130°-131° (0.25 mgm) (108, 109)
- Diallyl 1,4-hydroquinone diacetate, m p 111°-112° (0.25 mgm) (108, 109)
- Diamylhydroquinone (5 mgm) (239)
- Naphthalene (100 mgm per kg diet) (16)
- 1,2-Dihydroxy anthraquinone (100 mgm per kg diet) (16)
- Dihydro-anthraquinone diacetate (5 mgm) (239)
- Anthraquinone B-sulfonic acid (5 mgm) (239)
- Phenanthraquinone (5 mgm) (239)

synthetic vitamin K₁, 2-methyl-3-phytyl-1,4-naphthoquinone, is highly active, but the corresponding 2-ethyl compound is inert (fig 2C). Although vitamin K activity may be related to a specific type of molecular structure, such relationship is not as yet entirely clear. However, Fieser (108) has made some interesting suggestions re-

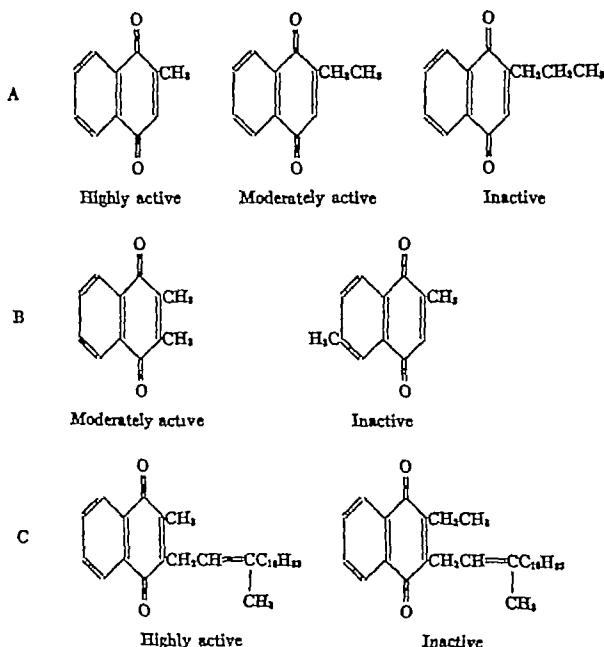


FIGURE 2

garding the specificity of the 2,3-dialkyl 1,4-naphthoquinones. He found that 2-methyl-3-cinnamyl and 2-methyl-3-geranyl-1,4-naphthoquinones, which have a total of 10 and 11 carbon atoms respectively in the side chains, have moderate vitamin K activity, while compounds with only 6 or 8 carbon atoms in these side chains were inactive.

The highly active synthetic vitamin K_1 has a total of 21 carbon atoms in these two side chains, and vitamin K_2 presumably has 30 carbon atoms. However, in this series of 2,3-dialkyl compounds, the simplest, 2,3-dimethyl-1,4-naphthoquinone, is moderately active, and thus occupies an anomalous position in this group. On the basis of the activity of this series of compounds, Fieser has postulated that the high vitamin K activity of 2-methyl-1,4-naphthoquinone may be due to the synthesis by the animal organism of vitamin K_1 from this compound and phytol. However, the data of Ansbacher, Fernholz and MacPhillamy (34) on the rates of utilization of 2-methyl-1,4-naphthoquinone and 2-methyl-3-phytyl-1,4-naphthoquinone offer no support for Fieser's hypothesis.

Natural occurrence of vitamin K

A large number of plants have been tested for the presence of vitamin K. Alfalfa (21, 22, 67, 72, 79, 129) and spinach (72, 79) are very rich in the vitamin. Other plants having a high natural content are cabbage (72, 134), cauliflower (72), kale (67), carrot tops (24), soy bean oil (25), nettle (72), chestnut leaves (72), pine needles (72) and seaweed (72). Less potent are tomatoes (67, 72), hemp seed (67, 72) and orange peel (67). Green leaves tend to be especially rich in the vitamin while leaves which grew in the dark contain much less vitamin K (24, 72). Also, fruits, seeds and roots contain considerably less vitamin K than do green leaves. The presence of large amounts of the vitamin in the chlorophyll-containing parts of the plant was one of the factors that led to the postulation (111) that vitamin K_1 contains, like chlorophyll, a phytyl group. Fieser (111) has suggested that K_1 arises in nature by the condensation of phytol and 2-alkyl-1,4-naphthohydroquinone, both of which occur in plants. Since both vitamins E_1 and K_1 contain a phytyl group, the possibility of a close biogenetic relationship between these two compounds has been pointed out (111, 149).

Bacteria likewise contain vitamin K (see p 354). Almquist, Pentler and Mecchi (20) determined the vitamin K content of a number of types of bacteria. Cultures of *Staphylococcus aureus*, *E. coli*, *B. subtilis*, *Sarcinia lutea* and *Mycobacterium tuberculosis* all contained abundant vitamin K. Approximately 0.6 to 2.0 gm of dried bacteria per kilo of basal diet protected against development of the disease,

as determined by clotting time measurements *Pseudomonas* and yeast developed very little of the vitamin. Some of these bacteria, then, are as potent as alfalfa, of which about 20 per cent in the deficient diets is required to prevent prolongation of the clotting time. The vitamin apparently is synthesized and retained within the bacterium during growth, as the culture media filtrate, free of bacteria, apparently contained none of the vitamin.

The growth of bacteria in putrefied fish meal undoubtedly accounts for its high content of vitamin K (22, 129, 188, 242). Feces likewise are rich in the vitamin, due, also, no doubt, to the bacterial content (23, 68, 125, 166).

Moderate amounts of vitamin K are found widely distributed throughout the animal body. It appears, however, that the vitamin present in the various organs of animals is of exogenous origin, and that animals are unable to manufacture the vitamin, except for bacterial synthesis in the intestine. Dam, Glavind, Lewis and Tage-Hansen (76) found complete absence of vitamin K in chicks kept on a vitamin K-deficient diet. In chicks on a normal diet, however, all of the organs tested were found to contain vitamin K. In their assays plasma, spleen, red muscle, gizzard, bone marrow and pancreas were found to contain relatively large amounts, while liver and lung were found to contain somewhat less vitamin K. Karrer (143) could not detect any vitamin K by chick assay in beef-retina extracts. In chicks on a diet rich in vitamin K, Dam and collaborators (76) found that the amount of vitamin in the organs was slightly increased. Increased stores of the vitamin with a high vitamin K diet is indicated also by the work of Almquist and Stokstad (23). However, even in those organs containing the greatest amount of the vitamin, their content appears to be less than 10 per cent that of alfalfa meal.

Although hog liver fat was one of the first sources of the vitamin to be recognized (66, 67), liver, whether from chicks, swine or dogs, contains relatively little vitamin K (23, 76, 79). Egg yolk contains small but variable amounts of the vitamin (23). Bile, which is necessary for the absorption of vitamin K from the intestine, has been shown, by assay, to contain practically no vitamin, provided bacterial growth is excluded. Beef, sheep and human bile have been tested (84, 124). Dam (68) tested the urine of a patient on a high vitamin K diet for the vitamin, but found none.

PLASMA PROTHROMBIN IN DISEASE

Simple dietary deficiency of vitamin K

1 *Birds* The deficiency in chicks has been described (see p 352) Dam, Schønheyder and Lewis (80) found that ducklings and goslings, placed on a vitamin K-deficient diet, developed a prolonged clotting time, and in a few of the animals hemorrhages occurred within 2-3 weeks Pigeons and canaries also developed the disease, but in a much milder form, and only after being on the deficient diet for several weeks

2 *Mammals* Many of the attempts to produce a hemorrhagic disease in mammals by feeding a diet deficient in vitamin K have failed It appears that in most cases there is absorption of sufficient vitamin, synthesized in the intestine by bacteria, to prevent the development of a deficiency Dam, Schønheyder and Lewis (80) fed young rats, guinea pigs, rabbits, dogs and swine a K-deficient diet, but they obtained no evidence of the disease, although this dietary regimen was continued for $2\frac{1}{2}$ months In a later study with rabbits, Dam and Glavind (74) did observe a mild deficiency as judged by their clotting test in one of three animals placed on a deficient diet Feeding a vitamin K concentrate corrected the clotting defect, and the deficiency reappeared in 28 days on the deficient diet alone In view of the single animal which developed the disease, one must consider that rabbits are quite resistant to the development of a simple dietary deficiency

Murphy (181) has given a brief report of a prolongation in the clotting time in mice fed on a purified diet Supplementing the diet with alfalfa extract prevented the development of the clotting defect No hemorrhages were observed, and no prothrombin determinations were made The picture was complicated by the presence of icterus If biliary obstruction were the cause of the jaundice, a vitamin K deficiency may also have been related to the obstruction However, the prevention of the condition by alfalfa supplements without the feeding of bile in addition, suggests that she was dealing with a true dietary K-deficiency of mild type

Greaves (125) has investigated very thoroughly the effects of a vitamin K-deficient diet fed to rats over a long period of time Only 12 of 77 rats (15.6 per cent) developed a deficiency sufficient to reduce

the prothrombin level to 50 per cent of normal or less. Many rats fed the diet for as long as 45 weeks did not develop a lowered prothrombin level, while others on the diet for only 6-8 weeks did. Rats of the third generation, on a vitamin K-deficient diet throughout, were no more susceptible to the disease than first generation rats. A possible explanation of these differences is that the amount of vitamin manufactured in the feces of the animals which became deficient was less, due to a difference in the intestinal bacterial flora. Individual differences in absorption might also be important. Even though the majority of these animals did not show significant decrease in their plasma prothrombin levels, their vitamin reserves were seriously depleted while on the deficient diet. This was shown by excluding bile from the intestine and thus decreasing or eliminating the absorption of the vitamin. Normal rats developed a severe vitamin K deficiency under such circumstances in approximately a month, whereas the animals maintained on a deficient diet prior to operation developed the hemorrhagic tendency in less than a week. Flynn and Warner (115) have obtained similar results.

Dam and Glavind (75) also have been able to produce a vitamin K deficiency in rats by feeding a vitamin K-deficient diet. As in Greaves' experiments, only a part of the animals (46.7 per cent) developed the deficiency.

A report of simple nutritional deficiency of vitamin K in man has been made by Kark and Lozner (142). They observed 4 patients with prolonged plasma prothrombin times, due apparently to a simple dietary deficiency. Three of the patients had been eating diets practically devoid of fruits and green vegetables for a period of one to nine years. In addition to the lowered plasma prothrombin, these patients had scurvy. The fourth patient suffered from chronic alcoholism, and had both pellagra and subclinical scurvy as well as a prolonged plasma prothrombin time. Vitamin K, without bile salts, was given by mouth to each of these patients. The day following the vitamin K therapy, the plasma prothrombin time of each of these patients was found to be normal or nearly normal.

Liver disease

Following experimental liver injury or hepatectomy, either partial or complete, there develops a profound fall in plasma prothrombin

(29, 198, 221, 249, 251, 255) A decrease in plasma prothrombin also occurs in certain types of liver disease Scanlon and collaborators (211) found that the prothrombin was reduced in each of their six cases of cirrhosis of the liver The prothrombin levels varied from 30 to 79 per cent of normal Stewart and Rourke (232) and Pohle and Stewart (190) likewise observed a reduction in the plasma prothrombin in hepatic cirrhosis In one of Pohle and Stewart's patients there was a hemorrhagic tendency Quick (198) noted in a patient with multiple liver abscesses a prolonged plasma prothrombin time (prothrombin 60 per cent of normal) Wilson (259) determined the prothrombin level in a group of 36 patients with liver damage, not associated with biliary tract obstruction or biliary fistula The prothrombin level was 80 per cent of normal or lower in approximately three-fourths of these patients Seven of the patients had prothrombin levels below 50 per cent of normal, and three were below 25 per cent

It appears that the hypoprothrombinemia in these patients is the result of the liver damage *per se*, and is not related to a vitamin K deficiency Therapy with vitamin K and bile salts in these patients was found to be without effect on the prothrombin level (211, 259) From their experiments with hepatectomy in dogs, Andrus and associates (29) concluded that the decline in prothrombin following hepatectomy is not influenced by peroral administration of vitamin K and bile salts In dogs with chronic liver injury induced by chloroform, we (49) have found that the fall in prothrombin is neither prevented nor cured by feeding large amounts of the vitamin along with bile salts

It appears possible that a hypoprothrombinemia may occur in patients having coexisting liver damage and vitamin K deficiency In this situation the extent to which the hypoprothrombinemia could be corrected by vitamin K therapy would appear to be limited by the functional capacity of the liver

The plasma prothrombin level appears to be a fairly sensitive index of liver function provided that there is not an associated vitamin K deficiency This was suggested by Smith, Warner and Brinkhous (221) in their study of plasma prothrombin in experimental liver damage in dogs Wilson (259) reached a similar conclusion from

a study of a group of patients with liver damage in whom he compared hippuric acid excretion (191) and the plasma prothrombin level. From his results, it appears that the plasma prothrombin level provides as good an index of liver function as does the amount of hippuric acid excreted.

Obstructive jaundice and biliary fistula

The bleeding tendency in patients having chronic biliary obstruction has been recognized for many decades, but in spite of extensive study of this condition, until recently there was no evidence which pointed conclusively to the clotting defect responsible for this hemorrhagic diathesis. The older work on this subject has been covered in a number of reviews, one of the most recent of which is that of Ravdin and Johnston (204).

The occurrence of a bleeding tendency in patients having chronic biliary fistula without jaundice was not appreciated generally in the past and, until recently, there were only a few reports of this complication. In 1935, the work of Hawkins and Whipple (131) with biliary fistula dogs served to emphasize the occurrence of a hemorrhagic diathesis in this condition. Animals with biliary fistula of several months duration frequently developed prolonged clotting times and hemorrhages, which at times were fatal.

Recent work from several different laboratories has shown that the hemorrhagic tendency associated with both obstructive jaundice and biliary fistula is due to the same fundamental causes. In each, the bleeding is due to a lowered plasma prothrombin level. This in turn is the result of a deficiency in vitamin K, which is absorbed inadequately from the intestine in the absence of bile.

1 *Prothrombin deficiency* The first studies of plasma prothrombin in patients having obstructive jaundice were reported in 1935 (192, 203). Quick, Stanley-Brown and Bancroft (203) observed a prolongation of the clotting time, as determined by their plasma prothrombin time test, in each of 5 cases of jaundice. In these patients their accelerated clotting time varied from 35 to 90 seconds in comparison to a normal value of 23 seconds. None of their cases showed a hemorrhagic tendency. These findings, they stated, "suggest that the hemorrhagic tendency in jaundice is brought about by a diminu-

tion of prothrombin Further work is required, however, before this conclusion can be definitely established "

Soon thereafter Hawkins and Brinkhous (130) reported prothrombin studies in chronic biliary fistula dogs At the time of spontaneous bleeding they found that the plasma prothrombin was at an extremely low level—less than 5 per cent of a normal control dog The whole blood clotting time, which was prolonged, was restored to normal by the addition of a partially purified prothrombin, free of calcium and thromboplastin Normal values for serum calcium and plasma fibrinogen were obtained, and there was no evidence of an excessive amount of antithrombin or of heparin These data showed definitely that the hemorrhagic tendency was the result of a prothrombin deficiency Bile feeding prevented as well as cured this bleeding tendency Further studies by Smith, Warner, Brinkhous and Seegers (222) showed that in these dogs the critical prothrombin level at which bleeding is likely to occur is approximately 10 per cent of normal Other studies have demonstrated that biliary fistulae, both in rat and in man, are complicated by a hemorrhagic tendency due to a lowered plasma prothrombin (Greaves and Schmidt (128), Warner, Brinkhous and Smith (252), Brinkhous, Smith and Warner (47), Greaves (124), and others)

Studies of the bleeding tendency in obstructive jaundice definitely indicated that it was due to a hypoprothrombinemia, thus substantiating the suggestion of Quick, Stanley-Brown and Bancroft Warner, Brinkhous and Smith (252) and Brinkhous, Smith and Warner (47) observed a marked lowering of the prothrombin in jaundiced patients with a hemorrhagic tendency No spontaneous bleeding was encountered unless the prothrombin had fallen to below 35–40 per cent of normal Furthermore, a rise in the prothrombin to above these levels was associated with cessation of bleeding

Dam and Glavind (70, 71, 74) observed high R-values (see p 359) in many of their cases of obstructive jaundice They have shown that the R-value is prolonged under a number of different circumstances The amount of calcium or fibrinogen, the pH, and the amounts of clot-inhibiting agents, as well as the prothrombin level, all influence the R-value They presented evidence, however, which indicated that the high R-values observed in jaundiced patients are

due to a deficient amount of prothrombin. They have found also that ligation of the common bile duct in chicks results in a high R-value in 24-26 days.

A number of other studies, including those of Butt, Snell and Osterberg (51, 52, 230), Stewart, with Rourke and Allen (231-234) and Pohle and Stewart (190) have all confirmed the finding of a lowered plasma prothrombin level in the bleeding tendency of obstructive jaundice.

The prothrombin level at which "cholemic" hemorrhage occurs has been studied in a number of human patients. Brinkhous, Smith and Warner (47) found that bleeding occurred only when the prothrombin level was below 35-40 per cent. However, no sharp prothrombin level could be considered as a bleeding level, as some patients with a plasma prothrombin as low as 20 per cent of normal have shown no evidence of hemorrhage. It appeared that aside from the prothrombin level, there were other factors, as trauma, infection, operative hemorrhage and exudate formation, which were important in inciting actual hemorrhage, and that these factors play a rôle in determining the exact prothrombin level at which hemorrhage occurs. Stewart and Rourke (232) studied six jaundiced patients at the time of massive hemorrhage, and found prothrombin levels varying from 13 to 36 per cent of normal.

On the basis of plasma prothrombin time determinations, there appears to be a wide range over which hemorrhage may occur. Quick (198) stated that the critical prothrombin level is approximately 15-20 per cent of normal. Butt, Snell and Osterberg (53) noted hemorrhages in patients with prothrombin times varying from normal to twice the normal value or higher, the prothrombin level was not calculated on a percentage basis. Pohle and Stewart (190) observed 6 patients with cholemic bleeding. The lowest prothrombin value in this group was 15 per cent of normal, the highest 35 per cent. Also, on the basis of prothrombin time determinations, Greaves (124) observed in rats with obstructive jaundice or biliary fistula that at the time of bleeding the prothrombin was at the 30 per cent level or lower.

In obstructive jaundice a lowered plasma prothrombin level, at least of moderate degree, is very common, as indicated by determina-

tions with the two-stage method Brinkhous, Warner and Smith (47) found that the prothrombin level was 80 per cent or lower in 23 of the 27 patients studied Stewart (231) observed a prothrombin level of 84 per cent or less in each of 17 patients in which jaundice had persisted for more than a week

Results as obtained by plasma prothrombin time (one-stage method) indicate a lesser incidence of hypoprothrombinemia in jaundiced patients Quick (198) stated that he observed normal prothrombin values in all but a comparatively small number of patients Butt, Snell and Osterberg (52) studied a number of patients with obstructive jaundice who had a normal prothrombin time, the exact frequency is not given Pohle and Stewart (190) found a normal prothrombin time in exactly half of their patients

These reported differences in incidence of hypoprothrombinemia may be due to the prothrombin method used It has been pointed out that if only undiluted plasma is used for determination of plasma prothrombin time, a practically normal prothrombin time may be obtained in the presence of only 60 per cent of the normal amount of prothrombin

2 *Vitamin K deficiency* That a vitamin K deficiency exists in cases of obstructive jaundice and biliary fistula was suggested for the first time in 1937 (197) on theoretical grounds The evidence then available indicated that (1) a hypoprothrombinemia exists in obstructive jaundice (192, 203), biliary fistula (130), and vitamin K-deficient chicks (81, 197, 215), (2) that vitamin K is a fat-soluble vitamin, (3) that certain of the other fat-soluble vitamins (A and D) require, as fats do, the presence of bile or bile salts in the intestine for their adequate adsorption (126, 127, 237), and (4) that the low prothrombin level and hemorrhages can be prevented or cured in biliary fistula dogs by feeding bile (130) It was clear that cases of bleeding in biliary fistula or in obstructive jaundice have in common the absence of bile in the intestine and that the bleeding tendency in each is due to a diminished plasma prothrombin On the basis of these facts, Quick (197) postulated, in addition, that they have a vitamin K deficiency, due to the lack of bile in the intestine

The first test of this hypothesis of a vitamin K deficiency was carried out in rats having a biliary fistula or obstructive jaundice by Greaves and Schmidt (128) in October, 1937 They confirmed the

earlier finding of Hawkins and Brinkhous (130) that bile feeding would correct the lowered prothrombin levels. In addition they found that feeding massive doses of vitamin K concentrate resulted in a prompt elevation of the prothrombin level. The question still remained whether bile itself might not contain the factor responsible for the prothrombin response. Further studies on biliary fistula dogs by Smith, Warner, Brinkhous and Seegers (222) showed that bile alone was much less effective in the elevation of the prothrombin level than a vitamin K preparation fed along with bile or bile salts. Vitamin K alone was practically without effect. The equal effectiveness of bile or bile salts indicated that the latter was the active material in bile (128, 222).

The first demonstration that vitamin K along with bile or bile salts is effective in overcoming the hypoprothrombinemia and bleeding tendency in human patients with obstructive jaundice was by Warner, Brinkhous and Smith (252) in January 1938. A vitamin K concentrate prepared from alfalfa, plus human bile or bile salts, led to a rise in prothrombin from 20-40 per cent to 85-105 per cent of normal in 6-8 days, whereas simple restoration of bile to the intestine required 2 weeks for a similar rise in prothrombin.

Similar beneficial effects of vitamin K in cases of obstructive jaundice were reported almost simultaneously by Butt, Snell and Osterberg (51) in February 1938 and by Dam and Glavind (70, 71) in March 1938. Butt, Snell and Osterberg (51) treated 18 cases successfully by using bile salt and vitamin K preparation of putrefied fish meal in a dosage 10 times greater than that required to cure the hemorrhagic chick disease. In the absence of bile, vitamin K was found to be ineffective in raising the plasma prothrombin, although bile alone, along with an adequate food intake, did cause a prothrombin response in 2 days. Dam and Glavind (70, 71, 74) gave a vitamin K concentrate intramuscularly for 3-4 days to 5 patients with obstructive jaundice, and found that the high R-values (5.6-88.0) present before treatment were returned to nearly normal values 1-3 days after the last dose. Similar results were obtained in young chicks with obstructive jaundice and high R-values (8-42). After intracardial injection of the vitamin concentrate, the R-values of the chicks were nearly normal one day later.

A fourth report on the use of vitamin K in obstructive jaundice,

by Quick (198) in May 1938, was less encouraging. His jaundiced patient had a marked reduction in the level of plasma prothrombin, but failed completely to respond to vitamin K therapy. Although subsequent reports by others have indicated that a few patients with jaundice fail to show a rise in prothrombin following vitamin K therapy, this is not the rule. The reasons for these occasional failures of response will be considered later.

Except for the report of Quick, other studies all confirmed the original finding of a prompt rise in the prothrombin level following vitamin K therapy in jaundiced patients. Brinkhous, Smith and Warner (47) reported 4 cases in which the prothrombin rose in 4-7 days from low prothrombin levels to normal or nearly normal levels with vitamin K and bile salt therapy. In 3 of these patients, the prothrombin level was at or near the bleeding level. With vitamin K therapy the prothrombin level reached 80 per cent of normal, and operative procedures were carried out. After operation there was no evidence of a bleeding tendency.

Other reports of the successful use of vitamin K in obstructive jaundice in 1938 were by Snell, Butt and Osterberg (230) and by Butt, Snell and Osterberg (52). In this work they used alfalfa as the source of the vitamin rather than putrefying fish meal, as in their earlier studies. They reported the successful treatment with vitamin K and bile salts of a large number of patients having obstructive jaundice and hypoprothrombinemia. Actual bleeding was controlled by this therapy in 13 patients.

In 1939 there were a large number of reports on the successful use of vitamin K therapy in the bleeding tendency and hypoprothrombinemia in patients with obstructive jaundice. Scanlon, Brinkhous, Warner, Smith and Flynn (211) observed a patient in whom cholemic bleeding could not be controlled by repeated blood transfusions, but which was controlled promptly by peroral vitamin K and bile salt administration. Rhoads (205) reported 12 patients, 9 of whom responded to vitamin K therapy, as judged by plasma prothrombin times. A dried preparation of young oat and wheat plants (cerophyl) was used as the source of vitamin K. Stewart (231) treated 12 cases of obstructive jaundice with vitamin K prepared from fresh spinach for 1 to 6 days. All showed a definite rise in

prothrombin Stewart, Rourke and Allen (234) found in 11 cases of jaundice due to calculous obstruction an average prothrombin value of 65 per cent which was raised to 96 per cent after $4\frac{1}{2}$ day treatment with vitamin K. Similar results were obtained in patients having obstruction due to carcinoma, the initial average pre-treatment value of 55 per cent was raised to 86 per cent after vitamin K therapy.

Other reports of the successful use of vitamin K in patients with obstructive jaundice were made by Olson (187), who treated one case, by Illingworth (140), who obtained a response in three of his four patients, by Caroli, Lavergne, Lavergne and Bose (55), by Olson and Menzel (186), who reported that 10 of their 11 patients showed improvement in the plasma clotting time, by Townsend and Mills (246), who observed a response in 9 of their 10 patients, by Koller and Wuhrmann (147), all of whose 10 patients responded to vitamin K therapy, and by Frank, Hurwitz and Seligman (117), who used synthetic vitamin K₁.

Additional reports on the continued successful use of vitamin K in obstructive jaundice were made by Butt, Snell and Osterberg (53, 54), Smith, Ziffren, Owen and Hoffman (223) with Flynn (224), Tage-Hansen (236) and Stewart and Rourke (233).

In addition to the studies of vitamin K deficiency in patients with obstructive jaundice, a number of similar studies have been made of patients with a biliary fistula. One of the first such cases reported (47, 252) had a complete external fistula and a hypoprothrombinemia and a bleeding tendency. With feeding of whole bile, the prothrombin level returned from a low level of 27 per cent of normal to 89 per cent. The vitamin K intake in the food was not determined, however. Successful treatment of the hypoprothrombinemia in these patients has been reported also by a number of investigators including Butt, Snell and Osterberg (52), Rhoads (205), Stewart (231), Stewart, Rourke and Allen (234) and Smith, Ziffren, Owen, Hoffman and Flynn (224).

Greaves (124, 125) has made a careful experimental study of the vitamin K deficiency which develops in rats with obstructive jaundice or biliary fistula. He studied the influence of feeding diets containing vitamin K and diets free of vitamin K on the development of the hypoprothrombinemia. He found that the presence or absence

of the vitamin in the diet did not affect the rate of fall of prothrombin postoperatively. From these studies it appears that the absorption of vitamin K from the diet is prevented practically completely by elimination of bile from the intestine. Treatment of the deficient rats, either with vitamin K concentrates plus bile or bile salts, or with large amounts of vitamin concentrates alone, was effective in raising the prothrombin level. Bile alone was ineffective provided the diet fed was free of vitamin K. This would suggest that the bacterial production of vitamin K in the intestine, although sufficient to protect against the disease in many cases, is not great enough to cure an established deficiency.

Zuckerman, Kogut and Jacoby (264) have reported an interesting experiment in a patient having a biliary fistula, with results similar to those obtained by Smith and collaborators (222) and Greaves (124, 125). They observed on feeding their patient a low fat diet, an increase in the plasma prothrombin time to 150 seconds. The coagulation time was prolonged, and spontaneous bleeding was observed. Neither a vitamin K concentrate, nor the patient's own bile, which drained from the fistula tract, administered separately by mouth for 4-5 days, was effective in curing the deficiency, but together they caused cessation of hemorrhage in 5 days, and after 8 days the plasma prothrombin time returned to normal.

3 *Postoperative decline in prothrombin level* It had been recognized for years that the bleeding tendency in obstructive jaundice is often observed within the first few days postoperatively. Although often there would be no evidence of excessive bleeding at operation, hemorrhage might be massive and fatal within the next few days after operation. Butt, Snell and Osterberg (52) described 8 patients with prolonged plasma prothrombin times and postoperative hemorrhage. The bleeding occurred in each case between the first and fourth postoperative days. In a few patients, hemorrhage was not observed until later—on the 18th day after operation in one (53).

The common occurrence of cholemic bleeding in the first few days postoperatively led to a careful study of the plasma prothrombin during this critical period. Butt, Snell and Osterberg (52, 53, 230) made the first extensive studies during this period. They observed a prolongation of the plasma prothrombin time with considerable

regularity in these patients following operation. Those patients in whom considerable liver damage in addition to obstruction of the biliary ducts was noted at the time of operation appeared to be most susceptible to postoperative hemorrhage. Stewart, Rourke and Allen (234) studied the plasma prothrombin level following surgery in a group of 19 patients with obstructive jaundice. Uniformly, they found a fall in prothrombin, despite the fact that a blood transfusion was given routinely at the time of operation. This fall averaged 20-25 per cent, and the minimum level was reached most commonly between the first and fourth days, coinciding, thus, with the time hemorrhage most frequently occurs. In two of their patients, the minimum level was reached on the 7th and 9th postoperative days, respectively. A comparison was made between the postoperative prothrombin levels in a group of four patients operated upon for biliary tract disease and a group of five patients without biliary tract disease or liver disease. These patients were followed for only the first 24 hours postoperatively. In the first group, a fall in prothrombin occurred with regularity, the maximum fall being 21 per cent. In the other group, a significant fall in prothrombin (15 per cent) occurred in only one patient. Ether anesthesia was used in the majority of these cases.

A number of causes for this fall in prothrombin after operation have been suggested. In one of the first patients treated with vitamin K preoperatively, Brinkhous, Smith and Warner (47) observed after operation a decline in prothrombin from a nearly normal preoperative level to a 20 per cent level on the tenth postoperative day. During this period the bile was drained externally, and the fall in prothrombin was attributed in this patient to lack of vitamin K absorption, due to absence of bile in the intestine. It appears that a number of other factors are important in determining this postoperative fall in prothrombin. Suggestions which have been made include (1) increased consumption of prothrombin due to hemorrhage and exudate formation, without adequate reserves of prothrombin or vitamin K to restore the prothrombin level promptly, (2) a low bile salt output by the liver and consequent malabsorption of vitamin K for a period after the obstruction has been relieved and (3) liver damage by anesthesia, infection, or operative trauma, and decreased production

(or increased consumption) of prothrombin by the liver. The importance of operative trauma to the liver as well as other tissues has been demonstrated experimentally by Lord (151). Simple massage of the liver in normal dogs for 25 minutes resulted in a prompt fall in prothrombin postoperatively in six of seven experiments. This postoperative level, which averaged about 70 per cent of normal, persisted for about 24 hours, followed by a gradual rise to a normal level. Similar massage of the spleen and kidney resulted also in a moderate decline in plasma prothrombin, but this hypoprothrombinemia persisted for less than 48 hours. Following operative trauma to the peritoneal surfaces in normal rats, however, Warner (249) observed no decline in the plasma prothrombin levels.

The rapid changes that occur in the prothrombin level postoperatively in patients with obstructive jaundice not treated with vitamin K may be due in large part to lack of adequate vitamin reserves, a suggestion made by many investigators. The dietary dyscrasia of these patients and the low fat intake, and probably more important, the faulty absorption of the vitamin preceding operation, account, no doubt, for the reduced reserves. Greaves (125) has found that rats on a vitamin K-free diet show a rapid fall in prothrombin after exclusion of bile from the intestine, while rats on a normal diet show a similar fall in prothrombin only after 3 to 4 weeks.

4 *Detection of potential bleeders* Since the hemorrhagic tendency in this disease is due to a profound lowering of the plasma prothrombin level, the detection of a potential bleeding tendency depends upon the plasma prothrombin determinations. Any of the methods used for the measurement of prothrombin, already described, appear to be satisfactory, provided the limitations of certain of these tests are recognized (see p 338, determination of prothrombin). If only the plasma prothrombin time of undiluted plasma is determined it appears that one may obtain a normal value in the presence of cholemic bleeding. Butt, Snell and Osterberg (53) have reported two cases in which this was noted. It appears from the results of many workers that a safe preoperative prothrombin level is approximately 75-80 per cent of normal. Because of the frequent and often profound fall in prothrombin postoperatively, frequent determinations of the prothrombin level during this period are desirable (53).

There are a number of other tests, aside from direct measurement of the plasma prothrombin level, which have been used, often successfully, for the detection of a bleeding tendency. These methods include R-value determinations (Dam and Glavind (74)), Ivy's bleeding time (141), clotting time of recalcified plasma (Olson and Menzel 186) and others), serum volume test (Boyce and McFetridge (42)), and coagelgrams (Nygaard and Baldes (185)). All of these procedures probably are influenced to a certain extent by the plasma prothrombin level of the blood, but a comparison between prothrombin levels and these tests have not been made except in the case of the R-value test (74) and the serum volume test (3).

5 Vitamin K therapy From the numerous studies on the vitamin K deficiency and hypoprothrombinemia in obstructive jaundice and biliary fistula, it is apparent that the bleeding tendency can be prevented, and in many cases it can be cured by treatment with vitamin K. To avoid the bleeding which may occur at the time of operation or soon thereafter, it is important to administer the vitamin for several days prior to operation, and to continue this therapy for several days after operation. The best guide to the need for vitamin K therapy appears to be the plasma prothrombin level of the patient. Hence frequent prothrombin measurements have been recommended. It has been suggested that even those patients with normal prothrombin values be given prophylactic doses of vitamin K (Butt, Snell and Osterberg (52)).

Many different sources of vitamin K have been used in the treatment of the vitamin deficiency in obstructive jaundice. Diets containing a large number of vegetables known to have a high vitamin K content (42) as well as synthetic naphthoquinones with vitamin K activity (54, 117, 146, 224) have been used.

All of the original vitamin K therapy was carried out by the use of relatively crude vitamin K concentrates. The choleic acid-vitamin K preparation of Almquist and Klose (11) was used with success by Cohn and Schmidt (59). This preparation would eliminate the need of separate administration of bile salts if the vitamin K is given by mouth. Successful use in patients of the synthetic vitamin K preparations, phthiocol (54, 224), synthetic vitamin K₁ (117), 2-methyl-1,4-naphthoquinone (146), 2-methyl 1,4-diacetyl naphtho-

hydroquinone (146), and 1,4-dihydroxy-2-methyl-3-naphthaldehyde (229) have been made

Viosterol was believed to be a value in checking or preventing the bleeding tendency in jaundice (43, 168) However, oral administration of vitamin D with bile salts in biliary fistula dogs (222) had no effect on the prothrombin level Wigodsky and Ivy (257) assayed a vitamin D preparation for vitamin K, and could detect none in it

The dosage of vitamin K that is required to give the best prothrombin response has not been studied in any exact manner As pure synthetic compounds are now available, and with the adoption of some standard unit system, this information should be forthcoming in the near future. The Iowa group (47) originally used the equivalent of 200-400 gm alfalfa meal daily Dam and Glavind (70, 71, 74) used a daily dose up to 45,000 Dam units intramuscularly Butt, Snell and Osterberg (52) used dosages equivalent to approximately 150-800 gm alfalfa From the assay of cerophyl, it appears that Rhoads (205) used the equivalent of about 20-100 gm alfalfa meal daily The dosage of the synthetic compounds has varied, depending on the route of administration and on the vitamin K potency of the compound Koller (146) used a single dose of 90-180 mgm of 2-methyl-1,4-naphthoquinone This compound as well as several of the other simpler forms of the 1,4-naphthoquinones appear to be somewhat irritating to mucous membranes Koller (146) gave four healthy persons 180-200 mgm of the 2-methyl compound No abnormality was noted except for nausea In rabbits a dose of 30 mgm per kilogram body weight produced no symptoms In dogs, however, in doses of 15-30 mgm per kilogram, given intramuscularly, 2-methyl-1,4-naphthoquinone caused vomiting, albuminuria, and porphyrinuria In mixtures of this compound and hemoglobin in vitro, methemoglobin formed In doses comparable to that given to patients (3 mgm per kilo body weight), no abnormality in dogs was noted Although thorough toxicological studies of these compounds have not been made, it appears that in amounts which are highly effective in correcting a vitamin K deficiency, the compounds are not toxic.

A number of different routes of administration of the vitamin have been tried Most of the data on vitamin K therapy for the hypoprothrombinemia of obstructive jaundice are related to peroral ad-

ministration This route has been highly effective except in those patients who were unable to retain the vitamin because of vomiting Butt, Snell and Osterberg (52, 53) have advised giving the vitamin and bile salts directly into the duodenum with a duodenal tube in certain cases with a greatly prolonged plasma prothrombin time or with active bleeding Stewart and Rourke (233) have performed a jejunostomy at the time of operation in certain cases and have used this route for the administration of the vitamin

With subcutaneous injections of vitamin K concentrates, Greaves (124) observed a rise in the prothrombin level in his vitamin K deficient rats In vitamin K-deficient chicks, however, Dam and associates (76) failed to obtain a response by this route with emulsions of vitamin K concentrates, unless the concentrates were heated first with desoxycholic acid

Intramuscular administration of vitamin K concentrates was used by Dam and Glavind (70, 71, 74) in their original studies of patients with obstructive jaundice The concentrate was given daily for 3-5 days, after which time the R-value was shortened considerably Similar results have been reported by Tage-Hansen (236) Koller and Wuhrmann (147) also used the intramuscular route The relative efficacy of this route and duodenal administration is shown in a case they reported Repeated intramuscular injections of a total of 500,000 Dam units of vitamin K failed to increase effectively the prothrombin level, as indicated by the plasma prothrombin time After the administration of 1,000,000 Dam units of the vitamin along with bile salts by duodenal tube, bleeding ceased promptly and the prothrombin time was nearly normal 14 hours later Butt, Snell and Osterberg (52, 53) have given the vitamin in peanut oil intramuscularly in a few cases With few exceptions, they observed no prothrombin response with this therapy From the data available, it appears that vitamin K concentrates are absorbed more slowly when given by intramuscular injection than by mouth In patients with a bleeding tendency, it would seem that the hematomas at the site of injection would interfere further with absorption

Intraperitoneal injection has been found to be an effective means of administering the vitamin in rats (115, 124, 125)

Intravenous administration of emulsions of vitamin K have been

used successfully by Dam and Glavind (74) in chicks with obstructive jaundice and high R-values, and by Tage-Hansen (236) in patients with high R-values. The first reported use of intravenous therapy of a known vitamin K preparation was by Smith and coworkers (223, 224) and practically simultaneously by Butt, Snell and Osterberg (54). Phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) was very effective in doses of 40–100 mgm. A rise in prothrombin was observed in less than 24 hours in all the cases studied. No toxic reactions have been reported. Snell and Butt (229) made similar observations with intravenous injections of 5–10 mgm of 1,4-dihydroxy-2-methyl-3-naphthaldehyde. This route appears to be ideal, especially in those cases in which there is nausea and vomiting, or in which there is interference with absorption from the intestine.

Smith and associates (224) stated that they observed no beneficial effect following the administration of the vitamin and bile salts with retention enemas.

It would seem that when more is known concerning the synthetic compounds having vitamin K activity and the relative effectiveness of various routes of administration, a better and more standardized mode of vitamin K therapy will be adopted.

Many cases of obstructive jaundice with a low prothrombin level respond poorly or not at all to vitamin K therapy. The failure of response to vitamin K in certain patients was recognized first by Brinkhous, Smith and Warner (47). In the patients to which they refer there was evidence of extensive liver damage which was responsible, at least in part, for the jaundice in these cases. As has been shown, liver damage will cause a hypoprothrombinemia without any obvious vitamin K deficiency. The experimental data available all point to the necessity of a liver in a good functional state for the production of prothrombin, and the failure of patients with liver damage to respond to vitamin K therapy is understandable. Greaves (124) observed that rats with obstructive jaundice, and presumably with some liver damage, responded more slowly to vitamin K administration than rats with a biliary fistula, which presumably have less liver damage. Butt, Snell and Osterberg (53) also have observed in patients with evidence of marked liver damage a delayed response, and in one case, no response to vitamin K therapy. How-

ever, they have noted many cases with severe liver damage who were able to maintain a normal prothrombin level with vitamin K therapy. In patients having a vitamin K deficiency complicated by liver damage, it appears that larger amounts of the vitamin are required for an increase in the prothrombin level (228, 234). There is no adequate experimental data available concerning this question.

Infection with complicating liver damage has been mentioned as a possible cause of failure to respond to the vitamin by Stewart (231) and by Greaves (125).

Other reports of patients with jaundice and a hypoprothrombinemia which failed to respond to vitamin K therapy have been made by Quick (198), Rhoads (205), Olson and Menzel (186) and others. Data are not available in these cases to know whether some of them would have responded to larger amounts of either the vitamin or the bile salts.

6 Blood transfusions In hypoprothrombinemia, the plasma content of prothrombin may be increased somewhat by blood transfusions (130, 152, 197, 222, 231, 234). Although the increase in prothrombin rarely exceeds 10 to 15 per cent, this may be sufficient at times to raise the prothrombin above the critical level, thus controlling hemorrhage temporarily. It would seem that, if available, a concentrated prothrombin preparation would be much more effective in raising the prothrombin level of the blood.

Hemorrhagic disease of the newborn

Low and variable plasma prothrombin levels in the newborn and in early infancy, and the presence of an extreme hypoprothrombinemia in hemorrhagic disease of the newborn were recognized in 1937 by Brinkhous, Smith and Warner (46). Since then, further studies on the low prothrombin level in normal infancy have indicated a possible relation to a vitamin K deficiency. These investigations in normal infants have contributed much to our understanding of hemorrhagic disease of the newborn, and they will be considered first.

The above workers (46) observed uniformly a reduction in the plasma prothrombin levels in the newborn and throughout infancy. Blood obtained from the umbilical cord at the time of birth showed considerable variation in the amount of prothrombin present. Values

varied from 14 to 39 per cent of the normal adult control value. During the first eleven days of life, values ranging from 26 to 44 per cent of normal were obtained. Thereafter, there was a gradual rise in the prothrombin until a nearly normal level was reached at the end of ten and one-half months. Maternal prothrombin determination, both antepartum and postpartum, showed no significant variation from normal control values. In a study of 50 newborn infants, low prothrombin values were reported also by McKhann and Edsall (167).

Owen, Hoffman, Ziffren and Smith (189) repeated the earlier work of Brinkhous and associates, and, in general, obtained the same results. They made more extensive observations during the first 10 days of life, and observed a transient fall in the prothrombin level between the second and sixth days of life. This fall, however, was not especially marked.

Hellman and Shettles (133) and Shettles, Delfs and Hellman (218) confirmed the finding of a low prothrombin level at birth. Cord blood, in their series, contained about 22 per cent as much prothrombin as the mothers' blood. Shettles, Hellman and Delfs (133, 218) also have studied a total of 17 premature infants and have found the average prothrombin level to be only about one-third that of full-term infants, that is, less than 10 per cent of the value of the mothers. No hemorrhages were observed in this group of premature infants, however.

A number of studies of plasma prothrombin in the newborn have been made, using the one-stage prothrombin time tests. Striking discrepancies were found in comparison to the results obtained by the two-stage test. Quick and Grossman (201) observed normal plasma prothrombin times in a group of 8 infants, 3 to 7 days of age. Later studies by Owen and associates (189), Quick and Grossman (202) and Waddell and Guerry (247) showed, however, that a variable and often marked prolongation of the prothrombin time occurs from about the first day through the sixth day of life. In general, however, a normal prothrombin time was observed at birth, and normal values were obtained again after the sixth day of life. Owen and associates (189) observed a prothrombin value as low as 15 per cent of normal on the sixth day as judged by plasma prothrombin

time, and a low value of 18 per cent on the fifth day by the bedside test. The lowest values observed by Quick and Grossman (202) were 7 per cent of normal, as determined by the plasma prothrombin time. Nygaard (184) confirmed the finding of a normal prothrombin time at birth and a variable and marked prolongation during the first to the sixth day of life. He observed a delayed prothrombin time as early as 10 hours after birth.

In their studies of clotting time in the newborn, Dam, Tage-Hansen and Plum (82, 83) observed a moderate increase in the R-values ($R = 1.3-8.0$) from the first to sixth days of life.

As indicated above, there are marked and constant differences in the plasma prothrombin level of infants, as obtained by the one-stage and the two-stage methods. These differences have not been explained completely. As has been pointed out previously (p. 336), a number of factors influences the conversion of prothrombin to thrombin, and any changes in the prothrombin conversion rate cannot be evaluated by the one stage prothrombin methods alone. In infancy there may be certain factors operating which allow a better utilization of the small amounts of prothrombin present. Changes in prothrombin convertibility and increased availability of thromboplastin have been suggested (189). It would appear, however, that any such mechanism is insufficient to compensate for the decline in prothrombin which occurs between the first and the sixth day of life, and with either the one- or two-stage methods, a fall in prothrombin during this period is observed.

With the finding of a normally occurring hypoprothrombinemia in infancy by Brinkhous, Smith and Warner (46), it appeared entirely logical that certain cases of idiopathic hemorrhagic disease in the newborn might well be the result of an exceptionally low prothrombin. This was indicated from their study of one case (46) during the period of hemorrhage, which occurred on the fourth to the ninth day. The prothrombin was extremely low—below 5 per cent of the normal adult value. The hemorrhagic tendency was completely controlled by transfusion and ten days later, the prothrombin was at the 32 per cent level, a normal value for infants of this age. Waddell, Gray, Bray and Kelley (248) and Waddell and Guerry (247) also reported a case of bleeding in the newborn. On the third day of life, there was

oozing from a puncture wound over a period of twelve hours and the prothrombin time of plasma was 6 minutes in contrast to normal values of less than 25 seconds. McKhann and Edsall (167) determined the plasma prothrombin time in three infants suffering from hemorrhagic disease of the newborn. The prothrombin values obtained were 5, 10-15, and 25 per cent respectively in these patients. Nygaard (184) has reported the largest series of patients with hemorrhagic disease of the newborn in which plasma prothrombin was studied. In his 9 cases, the plasma prothrombin time varied from 50 to 110 seconds, exceeding in each case the normally prolonged prothrombin time of infants of the same age. Hemorrhages and the delayed prothrombin times were observed in each case between the 10th and 120th hours of life. Rhoads (205) also studied a case of hemorrhagic disease of the newborn and found the prothrombin time to be 600 seconds. Thus, in each of these 15 cases of hemorrhagic disease of the newborn, there has been evidence of a low plasma prothrombin level.

The recognition of the rôle of vitamin K in prothrombin production led to the study of a possible relationship between this vitamin and the hypoprothrombinemia of the newborn. The first such studies were made by Waddell, Guerri, Bray and Kelley (248) and by Waddell and Guerri (247). The latter authors found that administration of vitamin K to a group of 10 normal infants eliminated completely the usual prolongation of the prothrombin time. Hellman, Shettles and Delfs (133, 218) also observed that the plasma prothrombin level is raised by feeding vitamin K to normal newborn infants. Treatment of one twin each of 4 sets of twins resulted in a prothrombin level of approximately one and one-half times that of the untreated twin. They observed in addition that a similar rise in the infantile prothrombin level could be obtained by administration of the vitamin to the mother either before or during labor. Nygaard (184) also has treated several newborn infants with vitamin K. In most of his cases, the usual delayed prothrombin time did not develop. Dam, Tage-Hansen and Plum (82, 83) treated with vitamin K two 4-day old infants having R-values of 8 and 4. Four hours after intravenous injection of vitamin K, the R-values were 2.5 and 2 respectively.

Waddell, Guerry, Bray and Kelley (247, 248), Nygaard (184) and Rhoads (205) have treated with vitamin K a total of 5 cases of hemorrhagic disease of the newborn in which actual bleeding occurred. In three of these cases in which the bleeding was external and in which vitamin K was the only therapy, hemorrhage ceased promptly and the plasma prothrombin time became normal or nearly normal in 2 to 10 hours after the beginning of the therapy.

In summary, a total of 15 infants diagnosed hemorrhagic disease of the newborn have shown in all instances a low quantitative prothrombin level or a prolonged plasma prothrombin time. This finding and the cessation of hemorrhages with a rise in the plasma prothrombin level indicate that the hypoprothrombinemia is the clotting defect responsible for the hemorrhagic tendency. Only 5 of the reported cases of hemorrhagic disease of the newborn have been treated with vitamin K. The prompt recovery following vitamin K therapy in these patients as well as the beneficial effects of the vitamin in normal infants indicate that a vitamin K deficiency is probably responsible for these cases of bleeding. However, there is a question whether the cessation of hemorrhage in the treated cases was merely coincidental with spontaneous recovery. The study of a larger number of patients should answer this question.

No experimental data are available which would indicate the reason for a vitamin K deficiency and the spontaneous recovery in the newborn. Deficient vitamin K reserves of the infant and an inadequate dietary source of the vitamin after birth have been suggested as possible causes. Jaundice appears to have no relation to the deficiency. Quick and Grossman (202) have pointed out that the recovery period coincides with the period in which the bacterial flora of the intestine develops. They have made the interesting suggestion that sufficient vitamin becomes available with bacterial growth to correct the deficiency. Whether or not any of these suggestions are of importance in the pathogenesis of this disease has yet to be determined by experimental study.

There appears to be a considerable difference in the prothrombin level at which hemorrhage occurs in the newborn and in adults. Brinkhous, Smith and Warner (46) found levels as low as 14 per cent of the normal adult value at birth without the occurrence of hemor-

rhage Similarly, Owen and associates (189) found in one patient a level of 6 per cent on the sixth day by the two-stage method, and Quick and Grossman (202) observed two 1-day old infants with prothrombin levels of 7 per cent, as judged by the plasma prothrombin time None of these patients showed a hemorrhagic tendency In contrast, similar low levels in adults have been associated uniformly with a hemorrhagic tendency Although a large enough group of infants has not been studied to determine the prothrombin level at which bleeding is likely to occur, it does appear that infants do not develop a bleeding tendency unless the hypoprothrombinemia is extreme. As pointed out above, some compensatory mechanism which insures the fullest use of the available prothrombin may be active in early infancy

Hemorrhagic sweet clover disease

A bleeding diathesis which develops in cattle following feeding of spoiled sweet clover hay was described originally in 1922 by Schofield (212) Subsequent studies on the development, clinical course, and morbid anatomy of this disease were made by Schofield (213), Roderick (208), and Roderick and Schalk (210) The disease is characterized by a prolonged whole blood clotting time and a bleeding tendency which is commonly fatal Hemorrhages in cattle usually occur only after the hay has been fed for about 4 weeks Young animals appear to be more susceptible to the disease than older ones Even though hemorrhages are not present, the animals with a prolonged clotting time are potential bleeders, and large economic losses have been suffered by dehorning or castrating animals in which the latent form of the disease was not recognized Rabbits and sheep are also susceptible to the disease, but it rarely occurs in horses

The nature of the clotting defect in this disease was studied by Roderick (209) He showed by a series of qualitative tests that there is probably a hypoprothrombinemia Prothrombin, prepared by Howell's acetone technique, reduced the clotting time to an approximately normal value either by mixing in vitro with the incoagulable blood or by intravenous injection Also, active prothrombin preparations could not be obtained from blood of the afflicted animals His studies of fibrinogen, calcium, clotting inhibitors and platelets showed no abnormalities

Quick (197) likewise observed a reduction in the plasma prothrombin in the experimentally produced disease in rabbits. When the prothrombin level, as judged by the plasma prothrombin time, was below 10 per cent that of normal rabbits, the hemorrhagic tendency developed.

Although the prothrombin deficiency in this disease appears to be well established, the mechanism by which toxic sweet clover depresses the prothrombin level is not well understood. Schofield (213) observed in the fulminating cases of this disease central liver necroses which at times were very extensive, involving a whole liver lobule. In chronic cases, fatty metamorphosis and hydropic degeneration of the hepatic cord cells were prominent. Roderick (208) also found liver necroses, but they were focal in distribution, and occurred only in about half of his cases. The liver changes are somewhat similar to those seen in acute and chronic chloroform intoxication, but whether or not the liver damage is sufficient to cause the hypoprothrombinemia in this disease has not been determined.

A possible relationship of this disease to vitamin K was suggested by Quick (197). From his experiments he concluded that small amounts of alfalfa in the diet were effective in preventing the disease or in arresting the progress of the disease. Ether extracted alfalfa meal appeared to be as effective as non-extracted alfalfa meal, a finding which would suggest that vitamin K, at least a fat soluble form, was not the material responsible for the protective action of alfalfa. However, Quick stated that the important means for controlling this disease is by giving a diet containing alfalfa. He suggested that the beneficial effect of alfalfa is exerted by supplying an accessory food factor required for prothrombin formation in the body.

Smith (225, 226) has made an extensive study in over 150 rabbits of the effect of feeding alfalfa on sweet clover disease. He was unable to confirm Quick's finding. Instead, he reached the conclusion that alfalfa exerted no influence, either prophylactic or curative, on this disease. He observed marked differences in the susceptibility of rabbits of similar age to toxic sweet clover. This work indicates the necessity of testing animals for susceptibility to the disease before using them in any experimental studies with toxic sweet clover hay.

From the data available, it appears that hemorrhagic sweet clover

disease is a toxic disease, unrelated to a vitamin K deficiency. However, there are no data which would indicate definitely the manner in which the intoxication affects the plasma prothrombin level.

Smith and Brink (227) have given evidence that the toxic material is elaborated in the hay during curing in a reaction involving coumarin, the compound responsible for the characteristic sweet smell of newly mown sweet clover and also in part responsible for the bitter taste of the clover. A non-bitter species of clover did not become toxic by the same curing process as did the bitter species of clover, and further, toxic alfalfa hay could be produced by adding coumarin to it before curing. The toxic alfalfa hay produced the same disease in rabbits as did the toxic sweet clover hay. Evidence indicates that coumarin itself will not produce the disease (210, 227). It is hoped that isolation of the toxic principle will allow a more careful experimental study of the mechanism of the prothrombin deficiency in hemorrhagic sweet clover disease.

Other conditions

1 *Sprue* Fanconi (97), in 1938, reported six patients with sprue who had a bleeding tendency and a prolonged coagulation time. Calcium and fibrinogen contents of the blood were found to be normal. He suggested that the bleeding tendency is related to the abnormal absorption of fats in this disease, and a vitamin K deficiency was postulated. However, Dam and Glavind (74) and Engel (96) found normal plasma prothrombin values in their cases of sprue. In later studies, Clark, Dixon, Butt and Snell (58), Butt, Snell and Osterberg (53) and Hult (139) found a moderate hypoprothrombinemia in a total of 4 cases of this disease. None of these patients exhibited a hemorrhagic tendency. The two cases reported by Butt, Snell and Osterberg had prothrombin levels, as determined by the two-stage method, of 47 per cent and of 62 per cent of normal. Hult's patient had an increased R-value (6.5 to 8.5).

Hult (139) treated his patient with vitamin K, given orally, for 4-5 day periods on several occasions. With this therapy, the R-value was reduced to a normal or nearly normal level, only to return to a high value when vitamin K administration was discontinued. The beneficial effect of vitamin K was noted both with and without ac-

companying bile salts, while a regimen with bile salts alone was ineffective

Although only a few cases have been studied, it appears that a hypoprothrombinemia, due to a vitamin K deficiency, occurs at times in sprue. Because of the relatively rare occurrence of a hemorrhagic diathesis in this disease, however, it would appear that a marked vitamin K deficiency is uncommon

2 *Other intestinal lesions* Clark, Dixon, Butt and Snell (58) and Butt, Snell and Osterberg (53) determined the plasma prothrombin levels in 13 patients having diverse intestinal lesions. Included in their studies were patients with internal and external intestinal fistula, chronic ulcerative colitis and intestinal obstruction. A marked diminution in plasma prothrombin was found in many of these patients, and in three, hemorrhage, due apparently to a low plasma prothrombin level, occurred. Administration of vitamin K corrected the hypoprothrombinemia. The vitamin K deficiency in these patients, they believe, was due to an insufficient amount of normal intestinal mucosa for adequate absorption. Certain other factors, as diarrhoea, long continued aspiration of bile and duodenal contents and toxic hepatitis, appeared to be important adjunctive causes of the vitamin deficiency and hypoprothrombinemia.

Stewart and Rourke (232) also have reported a reduction in the plasma prothrombin in patients with chronic ulcerative colitis. Treatment with a vitamin K-cholic acid mixture resulted in a gradual elevation of the prothrombin level.

3 *Artificially induced fever* Wilson and Doan (260) have studied the hemorrhagic tendency which occasionally occurs following artificial production of fever. Following hyperpyrexia, they found in all instances a reduction in the number of blood platelets. In addition, a few of the animals showed a decrease in the amount of prothrombin and fibrinogen, due apparently to liver necroses. Similar findings were noted in their patients following induced fevers, except that the fibrinogen was normal in all cases. Hypoprothrombinemia was most marked within the 24-hour period following fever therapy. The hemorrhagic diathesis in this condition is probably not due to a hypoprothrombinemia alone, since a thrombopenia was invariably present also.

4 *Anemia neonatorum, icterus gravis neonatorum and congenital hydrops* R-values in six infants belonging to this group were determined by Dam, Tage-Hansen and Plum (82, 83) The patients varied in age from one day to 26 days In all instances the R-value was high, it was over 300 in four of these patients In two of the three patients treated with vitamin K there was a prompt return of the R-value to approximately normal

5 *Hemophilia* In hemophilia the prothrombin content of the plasma is normal in amount (44, 71, 74, 203, 211, 232) With a normal plasma prothrombin level, one might expect that vitamin K would be ineffective in reducing the clotting time or in controlling the hemorrhagic tendency in this disease The reports of Dam and Glavind (74) and of Scanlon and associates (211) have indicated this Cheney (57) obtained divergent results by intramuscular injections of the vitamin in one patient

It is well established that the delayed clotting of hemophilic blood is associated with the slow conversion of prothrombin to thrombin (44, 91) and it has been suggested that there is a qualitative defect in prothrombin which is responsible (1, 91) However, no rigid experimental test of this hypothesis has been possible Brinkhous (44) found that addition of extremely small quantities of thromboplastin caused the prothrombin in hemophilic blood to be converted into thrombin at a normal rate, a fact which offers no support for the theory of a qualitative defect in prothrombin in this disease

6 *Miscellaneous* Certain other diseases in which spontaneous hemorrhages occur—acute leukemia, thrombocytopenic purpura and aplastic anemia—have been found to have a normal plasma prothrombin content (74, 211, 232) A normal prothrombin level in the presence of a low platelet content of the blood gives indirect support to the evidence previously cited that platelets are not the source of prothrombin

Moderate reduction in the prothrombin level (60–90 per cent of normal), but without a bleeding tendency, has been reported by Stewart and Rourke (232) in such diverse conditions as chronic lung abscess, rectal carcinoma and malnutrition, chronic duodenal ulcer, and melanoma The basis of such moderate hypoprothrombinemia has not been determined

In normal animals, there is prompt restoration of the plasma prothrombin level to a normal value following repeated bleedings. Even the removal of very large amounts of blood, either by repeated hemorrhages or by plasmapheresis, fails to reduce the plasma prothrombin level (251). Also, no marked changes in the prothrombin level were observed in incoagulable "peptone plasma" or "India ink plasma" (251).

Although the fibrinogen is increased greatly in dogs with experimental abscesses or distemper, the prothrombin level remains normal (251).

It has been suggested that there may be some relationship between menorrhagia and metrorrhagia and vitamin K. Wiles and Maurer (258) have prepared an unidentified lipid from liver which is stable to saponification and which, they state, is effective in the control of uterine bleeding. Although no prothrombin studies nor vitamin K assays have been done, they have suggested that this preparation is similar to Lichtman and Chambers' liver sterol (150), which has been reported to possess vitamin K activity. A case of multiple retinal hemorrhages which improved following administration of a hemp preparation having vitamin K activity has been reported (50). No prothrombin determinations were made.

Mason and Smith (162) found a marked prolongation of the plasma prothrombin time in chicks depleted of vitamin A and in chicks fed the pellagra like "T" ration of Ringrose, Norris and Heuser. In spite of extreme prolongation of the plasma prothrombin times (over 5 minutes), the whole blood clotting times were normal and there was no evidence of a hemorrhagic tendency. The influence of vitamin K on the clotting disturbance was not tested.

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RHEUMATIC HEART DISEASE PATHOGENESIS AND ETIOLOGY IN THEIR RELATION TO THERAPY AND PROPHYLAXIS¹

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A disease may be considered primarily from the viewpoint of an individual or from that of a group. The first is the one assumed between the physician and patient, the second embraces the relations between society at large and its constituent members who are afflicted with the malady under consideration. Each disease presents its own particular problems both in individual diagnosis and therapeutics and in possible provision for mass treatment and prophylaxis. Indeed prophylaxis may embrace not only the elimination of a disease, but the prevention or diminution of late sequelae or of untoward complications, for example, we now consider that effective therapy early in syphilis probably obviates late cardiovascular disease, tabes dorsalis and paresis. When any disease assumes such numerical proportions that society must provide for its victims, then society will probably demand that prophylactic measures be attempted.

On the other hand, the nature of the problem of an individual or of a group of individuals can be understood only by careful study of the life history of the disease under discussion, by the analysis of numerous cases and by much thought concerning the manner in which the observed phenomena may be altered. Even though the causation of a disease may not be entirely clear, it is important to study its course, and to discover factors which influence amelioration or recovery, in contrast to those which lead to physical deterioration or death. It now is generally admitted that multiple causative factors are operative in the pathogenesis of many diseases, and particularly does this seem to be true in rheumatic fever and rheumatic heart disease. For an

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intelligent understanding of either the physician's or society's attitude towards this disease a clear contemporary picture of pathogenesis and etiology are requisite

PATHOGENESIS

The noxious agent injures primarily the supporting structures of the body, viz, the collagen and elastica. Microscopic evidence indicates that usually each focus of injury is minute, but that the total number of foci may be myriad. The mildest injury appears to be a swelling of the ground substance between the fibrils (so-called early



FIG 1 FOCAL FIBRINOID DEGENERATION IN MURAL ENDOCARDIUM OF PATIENT A L WHO DIED ON 10TH DAY OF THIRD ATTACK OF RHEUMATIC FEVER $\times 100$

infiltrate or fibrinoid degeneration, 1 (see Fig 1)), the severest injury leads to actual necrosis of the fibres (see Fig 2). The immediate response to these injuries is inflammation with certain peculiarities. For a variable but relatively temporary period the classical features of edema, redness, local heat and acute pain in certain structures indicate the exudative nature of the reaction. Bedside observation demonstrates the large amount of simple edema fluid that can accumulate in the tissues, aspirated synovial fluid contains many wandering cells such as granulocytes, mononuclears and microphages, and in biopsy sections these cells are found throughout the involved structures (see Figs 3 and 4). It is therefore obvious that the exudate is often much

more massive and extensive than the demonstrable focal injury to the collagen, and the tumefaction may render the involved tissue more vulnerable to other injurious influences

An almost pathognomonic peculiarity of articular and periarticular rheumatic exudates is the manner in which they disappear under adequate dosage of certain antipyretic drugs. Moreover, these striking exudative features may be absent later in the disease when proliferative manifestations indicate continuing or renewed injury to the



FIG 2 SINGLE VERRUCA ON AORTIC VALVE OF PATIENT F. C. WHO DIED UNDER ANAESTHESIA ON THE 19TH DAY OF THE FIRST ATTACK OF RHEUMATIC FEVER

Note the small area of focal necrosis in tissue under the verruca $\times 170$

tissues. It seems as though enough immunity or resistance were set up to lessen the severity of the tissue reaction but not to obviate it completely. That this altered type of response is no figment of the imagination is proved by the appearance of subcutaneous nodules (see Fig 5) without any demonstrable macroscopic edema, or by the occurrence in the chronic type of disease of small areas of pleurisy or pericarditis without a serous exudate. Moreover, these predominantly proliferative lesions may appear when the patient is receiving large doses of antirheumatic drugs and nodules excised from a patient under such drug therapy contain extensive areas of fibrinoid degeneration.

It, therefore, is important to realize what our medicaments do and do not accomplish, and not to rely on them to the exclusion of other important therapeutic measures

The rheumatic granuloma, which in the heart is represented by the Aschoff body (see Fig 6), and in the subcutaneous nodule by a conglomeration of submiliary nodules (see Figs 6 and 8), is made up of

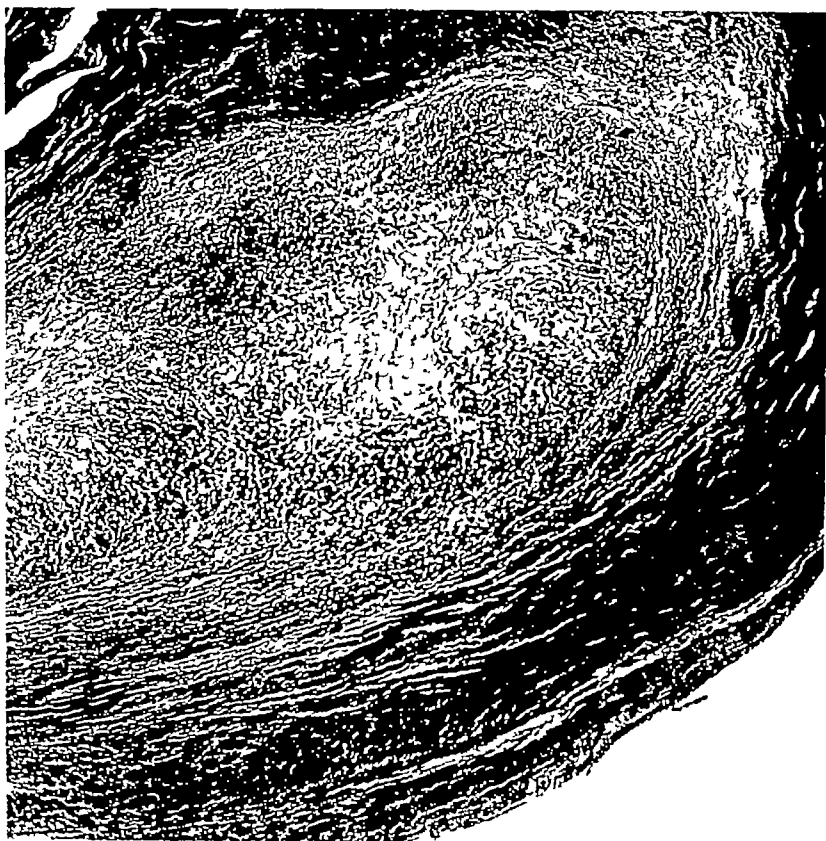


FIG 3 DIFFUSE EDEMA AND SLIGHT CELLULAR INFILTRATION IN AORTIC VALVE OF PATIENT F C (SEE FIG 2) $\times 100$

a mass of proliferative cells which have probably arisen from primitive resting mesenchymal cells (2) It is the peculiar arrangement of these cells which gives the submiliary granuloma, the Aschoff body, in the heart its typical appearance, in subcutaneous nodules they are less regularly arranged, in the heart valves, endocardium and larger blood vessels they are still more bizarre both in shape and distribution

Doubtless the number and arrangement of these cells in any given area are conditioned on one hand by the intensity of the injury, and on the other by the amount and architecture of affected collagen and elastica. Therefore what seems at first glance like a wide morpho-

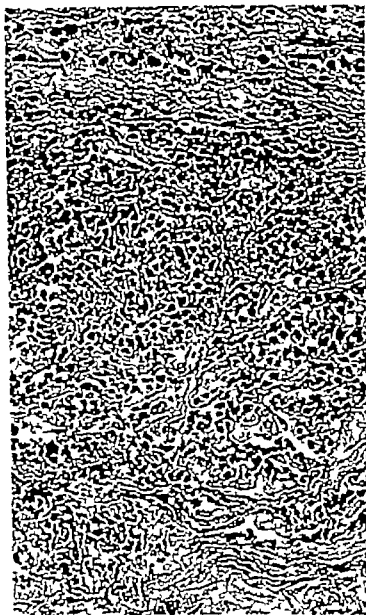


FIG. 4. INTENSE INFILTRATION OF POLYMORPHONUCLEAR CELLS IN MITRAL VALVE OF PATIENT H. B., WHO DIED OF HYPERTYREXIA ON THE 14TH DAY OF HIS SECOND ATTACK OF RHEUMATIC FEVER. $\times 300$

logical variety of lesions can all be reduced to a comparatively simple pathological entity.

The granuloma cells are gradually transformed into fibroblasts which lay down varying amounts of scar tissue, and thus in the healed areas there is finally much more collagen than existed originally (see Figs. 10 and 11). This increase of a substance which is especially

or covered, in the case of the heart, with mesothelium. The heart may be regarded as a modified part of this vascular tube, which, because of its anatomical and physiological peculiarities, is rendered

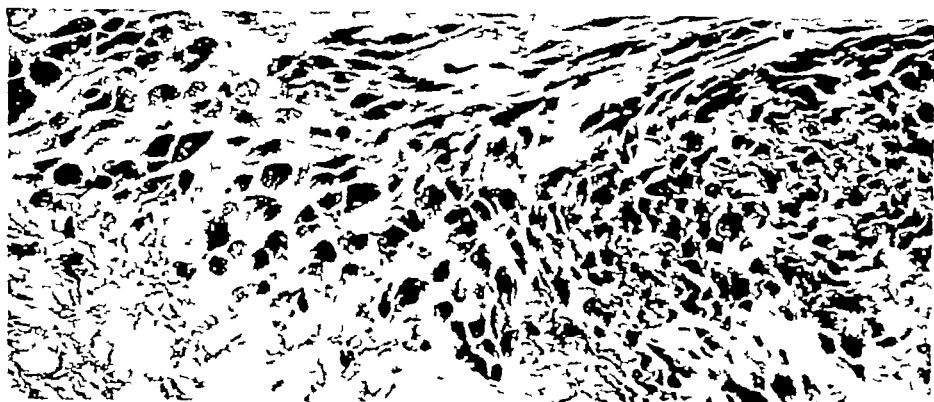


FIG 8 RHEUMATIC GRANULOMA IN THREE-WEEK-OLD SUBCUTANEOUS RHEUMATIC NODULE $\times 270$



FIG 9 RHEUMATIC GRANULOMA IN MITRAL VALVE OF PATIENT A B, WHO DIED ON 15TH DAY OF SECOND ATTACK OF RHEUMATIC FEVER

Note how the endocardium is broken over the granuloma $\times 100$

specially vulnerable to the "rheumatic poison." Numerous investigations within the past two decades have established the widespread involvement of the blood vessels in rheumatic fever, and the coronary

arteries and veins seem to share specially in this process (see Fig 12) Because excessive motion and functional strain are apparently important elements in localization of the rheumatic injury, both the heart and its blood vessels would appear to be peculiarly vulnerable. Numerous postmortem examinations on subjects dying in the first two or three weeks of an attack of rheumatic fever have now established the earliness of extensive lesions (see Figs 1, 4, 9-13). In these cases the fatal outcome was an index of the severity of the attack, but in

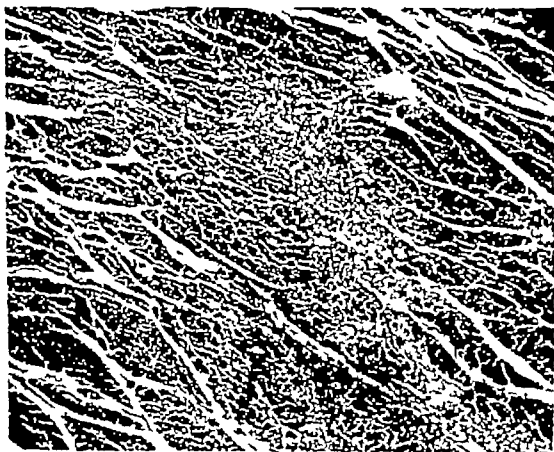


FIG. 10. EXTENSIVE SCARS IN MYOCARDIUM OF PATIENT L. S., WHO DIED OF CARDIAC FAILURE EIGHTEEN YEARS AFTER FIRST ATTACK OF RHEUMATIC FEVER. $\times 55$

the heart of one fever-free patient who succumbed to an anaesthetic on the nineteenth day after the onset of an apparently mild monocyclic course, there were edema and cellular infiltration of the aortic valve, early mild interstitial inflammation of the mitral valve, and splitting of the elastica with early mural thrombus formation in the coronary artery (see Figs 2, 3 and 12). Only one small verruca was found on the aortic valve with a very small necrotic focus underlying it. This unique case and also similar findings in subjects of accidental deaths in whom rheumatic fever was not suspected during life demonstrate

how insidious may be the development of the lesions (3) Moreo the careful microscopic examination of all of the valves of pati dying of rheumatic carditis has established the presence of infla mation in valves, especially of the right side of the heart, w appeared normal macroscopically (4) There is a considerable b



FIG 11 MARKED THICKENING OF AORTIC VALVE, PATIENT H M (FIG 7) Verruca formation at site of impact Many new formed blood vessels under vent lar aspect of valve Granulomata in valve ring $\times 12$

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fibrous structures forming their attachments so intimately are they related, and so incessant is their movement

This raises the question of the reason for the greater frequency and severity of verruca formation and permanent thickening of the mitral and aortic valves. It can best be explained by the greater amount of functional trauma to which these two valves are subjected. In the following order mitral, aortic, tricuspid and pulmonic valves, respectively, are the stress and strain to which they are submitted progressively reduced, and in the same order are they the bearers of verrucae and the sites of scarring deformities. Moreover, in the hearts of patients who have had long standing mitral disease with heightened

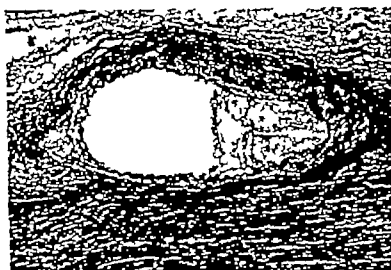


FIG. 12. EARLY ENDARTERITIS IN CORONARY ARTERY OF PATIENT F. C. (SEE FIG. 2) (FLASTICA STAIN) $\times 50$

pulmonary arterial pressure, and finally have suffered an acutely fatal attack of rheumatic fever, verrucae are much more frequently found on the tricuspid valves than they occur on the tricuspid valves of those subjects who have succumbed relatively early. In these patients with long standing heart disease the hypertrophy of the right side of the heart has doubtless created dynamic conditions in that side of the heart comparable to those normally existing in the left side and thus the physiological trauma of the inflamed valves is much increased.

The influence of increased work or trauma on the localization of cardiac lesions has recently been strikingly illustrated experimentally by Pearce (8) in rabbits infected either intravenously or intratesticularly with virus III. Without trauma only small cardiac lesions were

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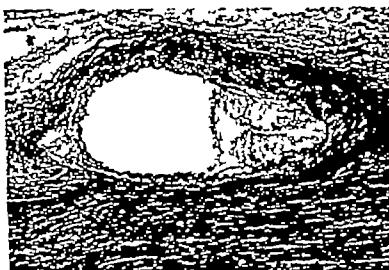


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induced, functional trauma following injection of gum acacia : resulted in a relatively large number of right heart lesions, injections were followed by relatively more left heart lesions, and punctures resulted in the highest proportion of pericarditis. experiments confirmed the previous observations of Clark and (9) and of Nedzel (10) concerning the influence of gum acacia and pitressin on the induction of endocarditis in animals infected with bacteria.

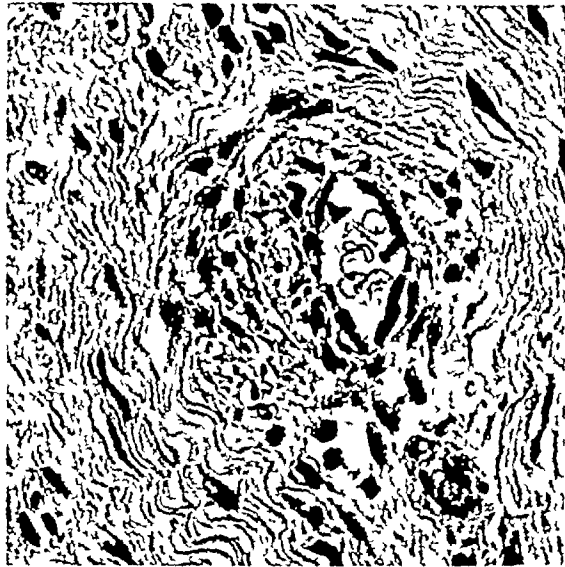


FIG 13 FRESH LESION IN A BLOOD VESSEL IN MITRAL VALVE

This vessel was probably formed as a result of a previous attack of rheumatic fever Patient A. B (see Fig 9) $\times 450$

With such evidence in mind, it is interesting to speculate upon the possible therapeutic effect of reducing the work of the left heart, in a patient with active rheumatic carditis, to that of the level of the right heart until the inflammation had subsided. Obviously, this is impossible, but some measures are applicable which might keep the demands upon the heart at the lowest possible level. For example the toxic and febrile condition accompanying rheumatic fever are both characterized by rather marked increase in the heart rate, and the mere fact of having active inflammatory lesions in the myocardium doubtless is a factor in accelerating the rate. Whatever other beneficial

effect is obtained from the use of antirheumatic drugs, the falling pulse rate that accompanies the disappearance of fever lessens the work of the heart to a considerable degree. For example, the reduction of ten beats per minute results in a total of 14,000 fewer per day. The



FIG. 14. ACTIVE CHRONIC VASCULITIS IN ILIAC ARTERY OF PATIENT J. S. (SEE FIG. 10)
X 80

physiological response to exertion is often accompanied by an acceleration of 20 to 40 beats per minute in patients with active or subacute rheumatic carditis. Reduced physical exertion, therefore, has a direct influence on diminishing the number of heart beats and saving the heart a material amount of damage.

A definite illustration of the influence of physiological trauma in conditioning localization of obvious reactions is seen in the joints where the arthritis is first manifest in rheumatic patients. In the greater number, the signs appear in the knees and ankles, joints most subject to physiological trauma, but in people, such as laundresses or seamstresses, who specially subject their hands and arms to stress, the localization often occurs earliest in these overworked joints. We have also seen instances where injury to a single joint was the starting point for a second bout of polyarthritis. If inflamed joints were kept in motion incessantly, it is highly probable that chronic deformities would occur in many patients with rheumatic fever. There is, in fact, a certain amount of experimental evidence indicating this possibility. For example, Vaubel (11) sensitized rabbits to horse serum and then injected this serum into one knee joint. Arthritis was induced in that joint alone when the animals remained quiet, but daily forced movements or very light percussion of the opposite knee, without any other injury, resulted in a chronic arthritis in the over-active joint.

The effect of physiological trauma in inducing an acute disease to become chronic is again illustrated in the work of Drs. Smadel, Farr and myself (12, 13, 14). Rats in which acute nephritis was induced with a specific nephrotoxic serum tended to recover when the dietetic nitrogen intake was reduced to a very low level. Many of the animals receiving even a normal amount of nitrogenous food eventually died of chronic nephritis, and all of those in which the nitrogen was at an abnormally high level developed fatal renal and vascular disease. Moreover, the rapidity of the development of this chronic disease varied in three different strains of rats. We thus had two variable factors: internal environment (i.e. amount of nitrogen handled) and hereditary susceptibility of the different strains. An important consideration was that even though the original trauma was acute and of short duration, the development of chronic disease was conditioned by the amount of work the kidneys were forced to perform.

Possibly the lessons learned from these experiments can be carried over to the problem of the rheumatic heart. In many instances, the inflammation resulting from the disease doubtless persists for months. If now, we consider that a structure, such as the heart valve, which is injured by an infectious agent is also subject to another kind of trauma,

physiologic in nature, it is not at all unlikely that the severity of inflammation and consequently the extent of resulting scar formation is much greater than would have resulted had that structure been kept absolutely at rest, or, in the absence of complete rest at the lowest possible level. It is perhaps not without significance that the verrucae form usually along the line of impact of the valvular leaflets, one on the other. Strain is also put on the chordae tendinae, which frequently are the sites of both verrucae on the surface and of inflammatory areas in the interior (see Figs 7 and 15). Thickening of these structures and fusion with their neighbors and with the mural endocardium, is one of the common reasons for marked deformity of the



FIG 15 FOCAL INTERSTITIAL VALVULITIS

Tricuspid valve of patient V I (See Fig 1). Most marked lesion at site of attachment of chorda tendinea. $\times 44$

mitral valves. It is also possible that the extensive motion to which the coronary blood vessels are subjected accounts in part, for the frequency with which these particular vessels are involved in this disease. Granted that prolonged rest is indicated as a therapeutic measure, it then becomes a public health problem to determine how that indication can be most effectively and economically met.

FACTORS OF INFECTION

While the exact manner in which rheumatic fever is induced is not as yet clear, the disease has all of the earmarks of being infectional in nature. At present, the hemolytic streptococcus seems to bear a

A definite illustration of the influence of physiological trauma in conditioning localization of obvious reactions is seen in the joints where the arthritis is first manifest in rheumatic patients. In the greater number, the signs appear in the knees and ankles, joints most subject to physiological trauma, but in people, such as laundresses or seamstresses, who specially subject their hands and arms to stress, the localization often occurs earliest in these overworked joints. We have also seen instances where injury to a single joint was the starting point for a second bout of polyarthritis. If inflamed joints were kept in motion incessantly, it is highly probable that chronic deformities would occur in many patients with rheumatic fever. There is, in fact, a certain amount of experimental evidence indicating this possibility. For example, Vaubel (11) sensitized rabbits to horse serum and then injected this serum into one knee joint. Arthritis was induced in that joint alone when the animals remained quiet, but daily forced movements or very light percussion of the opposite knee, without any other injury, resulted in a chronic arthritis in the over-active joint.

The effect of physiological trauma in inducing an acute disease to become chronic is again illustrated in the work of Drs. Smadel, Farr and myself (12, 13, 14). Rats in which acute nephritis was induced with a specific nephrotoxic serum tended to recover when the dietetic nitrogen intake was reduced to a very low level. Many of the animals receiving even a normal amount of nitrogenous food eventually died of chronic nephritis, and all of those in which the nitrogen was at an abnormally high level developed fatal renal and vascular disease. Moreover, the rapidity of the development of this chronic disease varied in three different strains of rats. We thus had two variable factors: internal environment (i.e. amount of nitrogen handled) and hereditary susceptibility of the different strains. An important consideration was that even though the original trauma was acute and of short duration, the development of chronic disease was conditioned by the amount of work the kidneys were forced to perform.

Possibly the lessons learned from these experiments can be carried over to the problem of the rheumatic heart. In many instances, the inflammation resulting from the disease doubtless persists for months. If now, we consider that a structure, such as the heart valve, which is injured by an infectious agent is also subject to another kind of trauma,

Epidemiology

Clinicians have long observed that rheumatic fever is frequently preceded by upper respiratory infections, notably tonsillitis or pharyngitis, which we know now are usually caused by hemolytic streptococci. During the first World War, Glover (23) studied the relationship of tonsillitis to the occurrence of rheumatic fever in young recruits in army training camps and noted that the same conditions of crowding that were accompanied by marked rises in meningococcus carrier rate seemed to play a role in raising the tonsillitis rate, which in turn was followed by the occurrence of many cases of rheumatic fever in these recruits. When suitable provisions were made for preventing the spread of streptococcal infections, both the tonsillitis rate and the incidence of rheumatic fever dropped practically to nil. More recently, Green (24) has made similar observations in groups of youths housed on British training ships. The frequent admission (weekly or fortnightly) of new recruits to these ships resulted in an almost constant occurrence of hemolytic streptococcal upper respiratory infections among the boys under training. The epidemiological phenomena resembled closely those described by Topley and Wilson (25) and by Webster (26) in studying paratyphoid infections in mouse populations to which new members were frequently added. When the admissions of recruits were spaced to three months intervals, the incidence of streptococcal infections diminished markedly and the rheumatic fever rate among these boys dropped correspondingly. Comparable observations concerning the relationship of rheumatic fever to tonsillitis and pharyngitis in English public school boys have been made by Glover and Griffith (27) and by Bradley (28), and in these studies, the types of hemolytic streptococci recovered were determined. In the above-cited epidemics the rheumatic fever in the majority of the boys was the first that these patients had experienced. There are now numerous reports of ward epidemics among previously rheumatic patients, or among patients who were convalescing from the recent acutely active disease. Collis (29), and Coburn and Pauli (30) have studied completely the relationship of group A hemolytic streptococci to these epidemics and have established quite definitely that this type of infection was by all odds the most prevalent. It is also evident from numerous reports that patients may suffer hemolytic streptococcal infections of the upper respiratory tract without immediately

more intimate relationship to the disease than any other known pathogenic agent. In recent years, the possible relationship of a filterable virus has been discussed. The first suggestive evidence was the reported agglutination of so-called "elementary bodies" by the serum of patients in the active stages of the disease by Schlesinger and co-workers (15) and Eagles et al (16). These "elementary bodies" were obtained by high speed centrifugation of rheumatic exudates. Recently, however, Eagles and Bradley (17) have demonstrated the non-specific nature of the reactions, because "elementary bodies" obtained from exudates of patients with several different arthritic diseases were agglutinable in the serum of patients with various types of arthritis. The possibility that pleuropneumonia-like microorganisms might have some etiological relationship in rheumatic fever (18) has now been fairly definitely disproven both by ourselves and several other investigators (19, 20, 21). In addition to the lack of direct demonstration of either a filterable virus or pleuropneumonia-like microorganisms in the blood, exudates or tissues of rheumatic fever patients, it has so far been impossible to induce, in any of the ordinarily employed laboratory animals, lesions closely resembling those of rheumatic fever, nor has the clinical course of the disease been reproduced in these animals. In order, therefore, to obtain some hints concerning the nature of a possible infectious agent we are forced to evaluate certain phenomena that occur during the period prior to the onset of an attack of rheumatic fever as well as others that take place during the course of the disease.

Here the study of hemolytic streptococcal infections in the patients seems to be of the greatest importance. It has now been definitely established that among the various groups of hemolytic streptococci described by Lancefield, the members of group A play, by far, the chief role in human infections (22). Moreover, members of the other groups, notably B and C, although frequently recovered from the upper respiratory passages of man apparently have little demonstrable pathogenic action in the carrier except in very rare instances. While cultural techniques may be valuable in differentiating the respective groups, by far the best method is that of immunological classification. It will be understood, therefore, that in the subsequent discussion group A hemolytic streptococci are the microorganisms considered in their possible etiologic relationship to the disease, rheumatic fever.

Epidemiology

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developing obvious rheumatic fever. Therefore, the entire mechanism and relationship is not entirely clear. For example, (31) at Irvington House, during the season 1937-38, among about 100 children convalescing from rheumatic fever, 14 had pharyngitis due to a single type of group A hemolytic streptococcus, and 10 of them developed rheumatic recurrences. In the following year, 35 similar children in the same institution had upper respiratory infections with hemolytic streptococci of different types, but none developed rheumatic relapses. In the same year, among 110 children of comparable age and severity of cardiac damage due to previous rheumatic attacks, who were living in their own homes, 11 per cent developed definite rheumatic relapses. The bacteriology in the latter group was not so carefully followed as in the institutional group. The low incidence or absence of rheumatic activity in the institutional group, however, suggests that the better physical condition of these children may have played a part in their higher resistance.

Immunological reactions

The course of events frequently observed has been described by Coburn and Pauli (32) as follows. Phase I, hemolytic streptococcal infection, Phase II, quiescent, and Phase III, rheumatic activity, it resembles closely that described by Escherich and Schick (33) in scarlet fever patients who subsequently developed nephritis. During these periods, certain immune reactions can be recognized. The one most discussed has been the rise in anti-streptolysin (anti-hemolysin) titre first observed by Todd (34). Subsequently, Todd (35) described a second hemolysin produced by group A hemolytic streptococci and its corresponding antibody. The earlier discovered one is now designated streptolysin O (oxygen labile), the second one, streptolysin S (oxygen stable). Most patients with hemolytic streptococcal infections, whether they develop rheumatic fever or not, develop the two types of antistreptolysins in their serum, and, as would be expected, the concentration of these antibodies varies within wide limits as does the duration of significantly high titres. Recently Todd, Coburn and Hill (36) have presented data, subjected to careful statistical analysis, which summarizes the reactions of several groups of their patients as follows. The O antistreptolysin titre was relatively higher with ac-

tivity of the rheumatic process and decreased as this disease improved, and the S antistreptolysin titre was comparatively low during the active stage of the disease and increased with recovery from the rheumatic symptoms. On the other hand, many workers who have studied the O antistreptolysin production by patients with rheumatic fever feel that its chief value is an indication that these patients are suffering from hemolytic streptococcal infections, indeed, no one with extensive experience with this phenomenon can doubt that these patients show as high a proportion of strong positive reactions as do a corresponding group of patients known to be infected with hemolytic streptococci but who were subsequently free from rheumatic symptoms. As this reaction is quite specific in its connotation, it can hardly be doubted that most patients with rheumatic fever are suffering from either active or recent hemolytic streptococcal infection.

The anti-fibrinolysin titre (37) of most patients with rheumatic fever is also increased in the same manner as in patients who have simple streptococcal infections. Recently, Boisvert (38) has noted that among rheumatic children a high antistreptolysin titre usually persisted over a longer period than among most nonrheumatic children suffering from simple hemolytic streptococcal infections. Prolonged persistence of such specific antibodies is suggestive that the stimulus to this antibody production, namely the hemolytic streptococci, is continually acting.

The antibody reactions above described are tested with antigens which are excreted into the media surrounding hemolytic streptococci. Other types of immune bodies against intracellular components of hemolytic streptococci have also been studied, namely anti P (nucleoprotein) precipitins, anti C (group specific carbohydrate) precipitins, and anti streptococcal agglutinins. None of these has been shown to have any significant differential relationship even though they occur in the serum of patients with this disease. On the other hand, the precipitin formation against the type specific component, namely M substance, has been shown to be somewhat different in rheumatics and non rheumatics. It is now established in mice and rabbits that antibacterial immunity in the case of group A hemolytic streptococcal infections is directly connected with the development of so-called anti M precipitins (39). Hodge and I (40) have compared anti M pre-

precipitin production by two groups of patients infected with hemolytic streptococci, namely rheumatic and nonrheumatic, in these studies the strain recovered from each individual patient was employed for making the type-specific M substance with which his serum was tested. The majority of nonrheumatic patients developed strong anti M precipitins by the fourth or fifth week. Among the rheumatic patients two trends were detectable. One group, in which short courses and fairly quick recoveries were the rule, developed high anti M precipitin titres early, the second group, characterized by long subacute courses of rheumatic fever, did not develop high anti M precipitin titres until late in the disease. Coburn (41) has reported similar differences between nonrheumatic and rheumatic patients infected with hemolytic streptococci. These findings suggest that possibly the prolonged persistence of the disease is related to low type-specific immunity against hemolytic streptococci.

Post mortem cultures

Still other evidence suggesting a peculiar action of hemolytic streptococci in the tissues of rheumatic fever patients has been recently advanced from postmortem cultures, in work of Green (42) and Collis (43). Green states that he recovered various types of group A streptococci from the valves or pericardium in eight out of nine subjects who had succumbed. The streptococci were recovered only from pathological valves or pericardial exudate, and not from blood cultures, and in five instances corresponded in type with strains isolated from throat cultures of the patients during life. Collis reported the recovery of hemolytic streptococci in eleven out of seventeen fatal cases, not only from the valves or pericardium, but also from peritonsillar tissues, mediastinal lymph nodes and spleen. Collis was very conservative in interpreting the significance of these post mortem cultures. Obviously, because of the numerous failures of others to recover similar microorganisms from rheumatic lesions, the findings of Green and Collis must be carefully checked by repeating their techniques, but their reports are of interest in connection with other evidence here discussed.

Chemoprophylaxis

Another test of possible relationship of streptococcal infections to rheumatic fever is the action of such an antistreptococcal agent as sulphanilamide. It has now been fairly definitely established that the drug has no curative action when given to patients with rheumatic fever (44), neither does it appear to prevent the disease in rheumatic subjects infected with hemolytic streptococci, if it is not given until after this infection has been established (45). On the other hand, Coburn and Moore (46) have recently reported that rheumatic children in the quiescent stages of the disease, who received the drug in 2 or 3 gram doses during the entire school year, apparently were protected from recrudescences of rheumatic fever. Among 184 subjects, who could tolerate the drug over this long period, practically none developed hemolytic streptococcal infections and only one developed rheumatic fever. Among a comparable group, not receiving this drug, 35 per cent developed hemolytic streptococcal infections of the upper respiratory tract and 20 per cent had rheumatic relapses. It is interesting that the drug could be tolerated over so long a period, and the observations certainly warrant repeating, but the well recognized toxic action of the sulphanilamide group of drugs indicates that such investigation should be carried out with the greatest of care in order to detect any toxic effects early and thus to obviate as far as possible serious drug complications.

While all of the foregoing evidence suggests strongly that there is a close connection between hemolytic streptococcal infections and rheumatic fever, it does not establish definitely the mechanism by which the peculiar course of events recognized as rheumatic fever is set up. The streptococcal infections may be merely the detonators which set off the explosions called rheumatic fever, which in turn may be actually due to some other infectious agent. On the other hand, the tissues of rheumatic patients may conceivably react in a peculiar manner to this bacterial infection. Partial, but not complete immunity of the rheumatic patient to these streptococci would explain how the microorganisms retain only a low grade irritative capacity in the tissues, and a reactive state of the tissues, called hyperergy, would account for the manner in which the tissues might respond to a very

small stimulus. In any event, from a public health viewpoint, protection of these patients from repeated hemolytic streptococcal infection apparently offers some hope of preventing recurrence of rheumatic fever. Especially are such prophylactic measures indicated in those subjects who have previously suffered from this disease and who are so liable to develop serious cardiac crippling if they experience recurrences.

SUMMARY

An attempt is made to describe the manner in which cardiac vascular damage develops as a result of rheumatic fever, and how the final picture results from either repeated insults to important tissues or from a long-continued low grade inflammatory process. Attention is directed towards the importance of functional trauma in localizing the permanent damage and scarring to certain structures, and to the role of this functional trauma in helping to continue an inflammatory process which might subside rapidly were complete rest attained. As a corollary, prolonged physiological rest is indicated to keep scarring at the minimum. The factor of infection in rheumatic fever is apparently closely related to the action of group A hemolytic streptococci, hence an important element in prevention of relapses is protection from such streptococci. A consideration of these factors is necessary either in handling a rheumatic individual or in framing a larger general program. Elsewhere (47, 48) are presented other features of rheumatic fever, such as the probable size of the problem and environmental influences which are amenable to alteration.

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THE MECHANISM OF HYDROGEN TRANSPORT IN ANIMAL TISSUES

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A discussion regarding the mechanism of hydrogen transport in animal tissues should perhaps be prefaced with the premises on which it is based. Among the major premises involved here are the following

- 1 That knowledge of certain of the chemical events which occur in living cells may be obtained from *in vitro* reconstructions of those events from definite chemical entities
- 2 That most biological oxidations are in reality the removal of hydrogen from the compound oxidized, as was suggested by Wieland (109, 122)
- 3 That the hydrogen of metabolites reaches oxygen in virtually every case through the intermediary action of certain vital catalytic agents (collectively referred to as biocatalysts in this discussion)

The first point does not imply that *in vitro* studies constitute the sole approach to the problem of biological oxidations. The results obtained with *in vitro* studies merely show what reactions *can* take place when certain specified reactants are mixed. It then remains for further studies under more nearly *in vivo* conditions to show what reactions actually *do* take place in the presence of the unknown biocatalysts which may effect side reactions in living cells. It is realized of course that the term *in vitro* covers a wide range of experimental conditions, ranging from experiments with surviving tissue slices to experiments in which the identity of every reactant is known.

With reference to the third point, the biocatalysts of tissue oxidations may be classified roughly as enzymes and as carriers. Since there is considerable disagreement as to the nomenclature and classification of these biocatalysts it becomes necessary to arbitrarily define the terms as used here. This discussion will be on the basis of hydrogen carriers, and remarks on the enzymes will be incidental to

their relation to the carrier compounds. A carrier compound will be defined as any compound which by virtue of its ability to be oxidized and reduced functions in the transport of hydrogen or electrons from tissue metabolites to oxygen. The carrier can exist in an oxidized form and in a reduced form, and it functions by oscillating between the oxidized state and the reduced state. Thus small amounts of a carrier can catalyze the transport of large amounts of hydrogen, and hence the oxidation of large amounts of metabolites. The proteins which are essential to the oxidation or reduction of the carrier compounds will be called enzymes¹. An example of a complete hydrogen transport mechanism is shown in Figure 1, which includes the known reactants

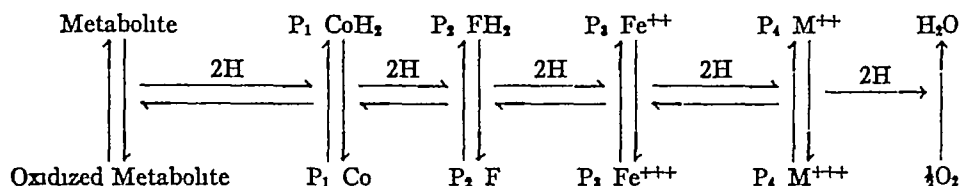
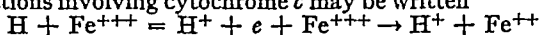
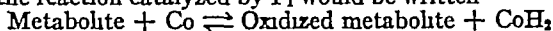


FIG 1 SCHEMATIC REPRESENTATION OF THE HYDROGEN TRANSPORT MECHANISM FOR METABOLITES WHICH REQUIRE COENZYME I OR COENZYME II

The horizontal arrows represent the path of hydrogen or electrons. P_1, P_2 , etc., refer to the proteins only. Thus P_1 = dehydrogenase, P_2 = protein of yellow enzyme, P_3 = protein of cytochrome c , P_4 = protein of cytochrome oxidase. Co represents coenzyme I or II, CoH_2 represents reduced coenzyme I or II, F represents flavin containing compound, FH_2 represents reduced flavin compound, Fe^{+++} represents prosthetic group of cytochrome c , Fe^{++} represents reduced prosthetic group of cytochrome c , M represents the hypothetical prosthetic group of cytochrome oxidase which presumably reacts with oxygen directly. Cytochromes a and b are not included since their status is still obscure. The reactions involving cytochrome c may be written



In chemical terms the reaction catalyzed by P_1 would be written



of a complete system for the oxidation of the metabolites which require coenzyme I or II. Most of the intermediates of carbohydrate metabolism fall in this group of metabolites. New catalysts may be dis-

¹ This concept of an enzyme is in conflict with that of the Warburg school (85, 117) but is in harmony with that of the Cambridge school (33) and furthermore is supported by recent experimental work (34) in which it was shown that various dehydrogenases (protein only) could remove hydrogen from their specific metabolite in the complete absence of the coenzyme previously considered essential, provided that certain non-specific easily reduced compounds were present in place of the coenzyme. Thus it appears that the enzyme is the protein and not the protein plus its coenzyme as was previously supposed. The observation is so new that confirmatory lines of evidence have not yet had time to appear, but if confirmed, this work will constitute an outstanding contribution to our knowledge of the mechanism of hydrogen transport.

covered later which will be found to serve as intermediates between some of the catalysts indicated here. However, it seems quite clear that P_1 in Figure 1 represents the various proteins which act as dehydrogenases.² The metabolite and the oxidized coenzyme (Co) appear to be adsorbed on the protein molecule in such a way that hydrogen passes from the metabolite to the coenzyme. The oxidized metabolite and the reduced coenzyme (CoH_2) then dissociate from the protein and the process can be repeated with more metabolite and Co molecules. It appears that the reduced coenzyme has a greater affinity for the protein P_2 than the protein P_1 . The protein P_2 has as its prosthetic group a flavin compound which functions as a hydrogen carrier. There appear to be several proteins analogous to P_2 and the flavin compounds are known in at least two types. In the presence of P_2 the two carriers react, regenerating the oxidized coenzyme (Co) and forming a reduced flavin compound, which although dissociable, has a dissociation constant many times smaller than that of the P_1 CoH_2 complex. We can consider the P_2 Fe complex as cytochrome c which is quite definitely a carrier in this system (51, 74, 90). If cytochromes a and b are involved in this reaction analogous complexes will have to be included in this scheme at the proper point. It has been pointed out by Hogness (53a) that the reduced flavin compound almost certainly does not dissociate from P_2 to reduce cytochrome c . In other words the reaction between FH_2 and Fe^{+++} appears to require both proteins P_2 and P_3 . This is indicated by the fact that the "old" yellow enzyme does not reduce cytochrome c , while a new yellow enzyme (51), having the same prosthetic group, will reduce cytochrome c very rapidly. The protein P_4 in Figure 1 represents the protein of cytochrome oxidase which probably possesses an autooxidizable prosthetic group at present unidentified (60). In the case of the reaction between Fe^{++} and M^{+++} it is to be assumed that both proteins P_3 and P_4 are required. It has been demonstrated experimentally that CoH_2I can react with the P_2 F complex in the complete absence of P_1 , and it appears that the coenzymes I and II

² The reviewer has resorted to the use of the letter P with various subscripts to refer to the various proteins or groups of proteins involved in order to save space in the text, when referring to the figures. It appears that there are several instances in which the reversible reduction of a given carrier may be catalyzed by more than one protein.

oscillate between proteins P_1 and P_2 as they oscillate from the oxidized to the reduced state. There is however no evidence that the remaining carriers oscillate in an analogous manner.

Figure 1 represents an attempt to integrate the present known facts into a scheme of hydrogen transport which will depict the path of hydrogen from metabolites to oxygen. It remains to be seen whether or not the path is as direct as indicated and to what extent "anastomoses" occur. Many compounds are known to be reversibly oxidized in cellular economy and hydrogen carrier function has been attributed to many of these compounds. It is here proposed to set up the various criteria which may be applied from an experimental point of view, in order to determine whether or not a given compound is a carrier in the mechanism of hydrogen transport, and then to apply these criteria to the various compounds which have been suggested to function as hydrogen carriers.

For the present the definition of a carrier as given above will be used regardless of the degree of dissociation which exists between the protein and the group which is reversibly oxidized and reduced. Included in this definition of carriers are the following: coenzyme I, coenzyme II, cytochromes *a*, *b* and *c*, riboflavin, and alloxazine adenine dinucleotide. In addition to these compounds the following will be considered since they have been suggested at one time or another as hydrogen carriers: Vitamin C, glutathione, adrenochrome, cocarboxylase, and various tissue metabolites. The latter will be considered in some detail since their discussion involves the Szent-Gyorgyi theory as well as the Krebs citric acid cycle.

Among the criteria of hydrogen carriers may be listed the following

- 1 The compound must be a natural constituent of animal tissues. All the compounds mentioned in this discussion satisfy this requirement.
- 2 The compound must be capable of being reduced by tissue preparations at a rate compatible with the rate of oxidation of the substrates whose oxidation it is presumed to catalyze.
- 3 The reduced compound must be capable of being oxidized by tissue preparations at an adequate rate as in point 2.
- 4 The compound must be capable of catalytically stimulating the rate of hydrogen transport in the system under investigation.

Rigid proof would require the preparation of a system free of the compound, which would function only if the compound were added

The above criteria have all been applied at one time or another to most of the compounds which have been suggested to have carrier function, but it now appears that it is possible for a compound to fulfill all of these requirements and still not function as a hydrogen carrier. In order to obtain a better understanding of the mechanism of action of these compounds as distinguished from carrier function, a fifth requirement has been devised, namely

- 5 The compound must be directly reduced by one system and directly oxidized by a second system which is not identical with the first

Since the last point does not seem to have been applied heretofore it seems desirable to state the reasons which led to its development.

Oxalacetate, as an example, occurs in tissue, can be reduced by tissues, and the reduced compound can be oxidized by tissues (107). Furthermore the compound produces increases in oxygen uptake greater than can be accounted for by the complete oxidation of the compound itself (99). Thus the first four requirements of a hydrogen carrier as listed above are met and it would seem from a casual examination of the facts that oxalacetate is indeed a hydrogen carrier. On the other hand, the fact that the compound produces increases in oxygen uptake beyond the amount required for its own combustion is easily explained by the work of Krebs (66, 68) who has shown that this compound condenses with pyruvate to effect the further oxidative breakdown of that compound by a cycle of reactions which regenerates oxalacetic acid. The fact that the compound can be reversibly reduced by no means proves that it is a hydrogen carrier, as is shown in Figure 2, which is a schematic representation of the Szent Györgyi theory using the system of notation employed in Figure 1. It is generally agreed at present that malic dehydrogenase and coenzyme I are required for the reduction of oxalacetate or the oxidation of malate (44, 107). Thus the oxalacetate malate system represents not a stage in the main channel of hydrogen transport, but merely a blind alley from which hydrogen cannot proceed toward oxygen except at the point it came in. That this is not merely an illusion can

be proved by chemical equations. If one considers the oxidation of CoH_2I by fumarate through the intermediation of the oxalacetate-malate system according to the Szent-Gyorgyi theory, the reactions may be written as follows

- (1) $\text{CoH}_2\text{I} + \text{oxalacetate} \rightarrow \text{CoI} + \text{malate}$
- (2) $\text{malate} + \text{CoI} \rightarrow \text{oxalacetate} + \text{CoH}_2\text{I}$
- (3) $\text{CoH}_2\text{I} + \text{fumarate} \rightarrow \text{CoI} + \text{succinate}$

A mechanism must exist for reaction (3), i.e. the oxidation of CoH_2I by fumarate, whether CoI was reduced by malate or by some other metabolite. Furthermore since reaction (2) is the exact reverse of reaction

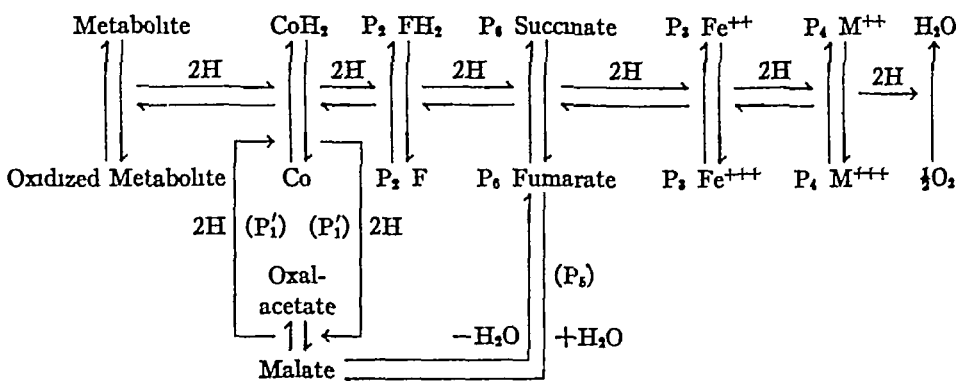


FIG. 2 SCHEMATIC REPRESENTATION OF THE SZENT-GYÖRGYI THEORY OF HYDROGEN TRANSPORT

Carriers and enzymes abbreviated as in Figure 1. In addition, P_1' represents malic dehydrogenase, P_2 represents fumarase, and P_3 represents succinic dehydrogenase. In the theory all three cytochromes are included although only c is shown here. (See 109, pp. 35-42.)

(1), no change has occurred in the energy content of the system and the net effect is that hydrogen has not been transported. (See also 13, 79, 88.)

The above points do not prove that oxalacetate is not a hydrogen carrier and they have not been introduced for that purpose. The points have been developed merely to show the need for a further type of experimental evidence to decide whether compounds such as oxalacetate function as hydrogen carriers. It is entirely possible that oxalacetate is a hydrogen carrier, and if so it should be possible to isolate two separate systems for its oxidation and reduction just as in the case of cozymase. The reviewer merely points out that thus far

this has not been done, and emphasizes that the first four criteria listed above fail to detect the weakness in the data which are used to show that any given compound is a carrier. As a matter of fact, many of the compounds which are generally accepted as carriers have not yet been shown to fulfill all five requirements and although they probably do function as hydrogen carriers, the mechanism of this action is still unknown. Cytochrome *c* is an outstanding example of this situation.

One is tempted to insist that a given compound be shown to be essential for a given reaction, but the possibility that alternate transport mechanisms exist seems to forbid the rigid application of such a requirement.

On the basis of the above five requirements of a hydrogen carrier, then, we shall proceed to examine the facts that are known with regard to compounds which might have carrier function.

COENZYME I

This compound is variously known as coenzyme I, cozymase, diphosphopyridine nucleotide and the abbreviations CoI and DPN. The reduced compound is referred to as dihydrocozymase, reduced coenzyme I and as CoH_2I or H_2DPN . Both coenzyme I and coenzyme II are interesting from the medical standpoint since their functional group is the amide of nicotinic acid, which has been found to be the anti pellagra vitamin (36, 98), and hence an explanation as to the function of the vitamin is at hand (35).

The compound is widely distributed¹ and may be specifically determined on the basis of its catalytic properties (7).

A number of metabolites are able to reversibly reduce coenzyme I in the presence of their specific dehydrogenases (P_1 in Fig. 1). Thus it has been shown that lactate (45), malate (44, 107), β -hydroxy butyrate (47), alcohol (76, 93), and glyceraldehyde diphosphate (6, 83, 120) all require coenzyme I for their oxidation in animal tissues, while glucose (27, 93), and glutamate (4, 39) apparently use CoI or CoII. Since most of these oxidations are reversible it is possible for one

¹ The references to the many reports dealing with the distribution and chemistry of coenzymes I and II and certain other coenzymes are given in the recent review by Baumann and Stare (21) and will not be duplicated here.

metabolite to be oxidized by the oxidized form of another metabolite of higher potential (48, 120), thus (see also Fig 3)

(4) glyceraldehyde diphosphate + CoI \rightarrow diphosphoglyceric acid + CoH₂I

(5) CoH₂I + pyruvate \rightarrow CoI + lactate

This type of reaction (dismutation) occurs in the absence of any of the biocatalysts which transport hydrogen from CoH₂ to oxygen or under anaerobic conditions, and it is probable that all of the metabolites which react with coenzyme I are in dynamic equilibrium with each other even under aerobic conditions. However it must be remembered that, so far as is now known, the metabolite which accepts hydrogen cannot be reoxidized except by a reversal of the reducing reaction, and then the net energy change becomes zero. When metabolism is proceeding, the reduced form of the various metabolites which take part in these dismutations are continually being formed and are consequently tending to reduce rather than to oxidize the coenzymes.

It is quite clear that reduced coenzyme I is oxidized in the presence of a protein corresponding to P₂ in Figure 1 and that this protein is distinct from the dehydrogenase which reduces coenzyme I. A number of enzymes of the P₂ type have been described in the literature and all have proved to be flavoproteins. These include the old yellow enzyme obtained from yeast by Warburg and Christian (118), which has riboflavin phosphate as its prosthetic group, and various other preparations obtained from animal tissues which have been suggested (3, 26) to be identical flavoproteins having alloxazine adenine dinucleotide as their prosthetic group. These include Straub's flavoprotein (106), diaphorase I of Euler et al (5, 3) and the coenzyme factor of Green and Dewan (26, 30, 46). Although none of these flavoproteins appear to be linked directly to cytochrome *c* there is evidence that either they are modifications of an active form which does react with cytochrome *c* (51) or that a third biocatalyst such as cytochrome *b* is required for the reaction with cytochrome *c* (74, 90). At any rate it seems clear that reduced coenzyme I is oxidized by a flavoprotein and that requirement 5 is fulfilled. In a recent paper by Dixon and Zerfas (34) the following statement is made "Cozymase acts as a coenzyme because it is not only reduced by the dehydrogenases but is then reoxidized by other systems (flavoproteins, etc)."

COENZYME II

This compound is known as Warburg's coenzyme, triphosphopyridine nucleotide, CoII, or TPN, and the reduced form is referred to as reduced coenzyme II or merely CoH_2II

Coenzyme II is widely distributed throughout the various animal tissues and has been isolated from red blood cells, from heart muscle, and from liver (21)

The reduction of coenzyme II in the presence of specific enzymes from animal tissues is effected by hexose monophosphate (Robison ester) (30), and probably by its breakdown products (31, 32), as well as by iso-citric acid (4) Coenzyme II is essential for the oxidation

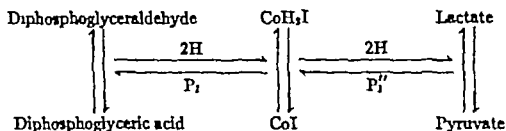


FIG 3 DISMUTATION OF GLYCERALDEHYDE DIPHOSPHATE AND PYRUVATE TO FORM DIPHOSPHOGLYCERIC ACID AND LACTATE

P_1 = glyceraldehyde diphosphate dehydrogenase.

P_1' = lactic dehydrogenase.

of these compounds Glutamic acid (4, 39) and glucose (27, 93) use either CoI or CoII as hydrogen carriers

Dismutations, or the oxidation of one metabolite by another, such as occur with coenzyme I, also appear to be possible in the case of coenzyme II Adler et al (4), using an enzyme preparation containing only proteins of the P_1 type, were able to effect reactions analogous to that shown in Figure 3 Thus iso-citric acid plus α iminoglutaric acid yielded α ketoglutaric acid and glutamic acid

The oxidation of reduced coenzyme II has been reported to take place in the presence of various flavoproteins of the P_2 type including Straub's flavoprotein (106), Dewan and Green's coenzyme factor (26, 30), and diaphorase II of Euler et al (2) Adler and Abraham (1) have reported that Straub's flavoprotein and therefore presumably the coenzyme factor of Green and Dewan do not oxidize CoH_2II In all of the above cases the flavoprotein was linked either to methylene blue or to oxygen Theorell (113) had reported cytochrome *c* re

duction with the old yellow enzyme and CoH_2II but the rate was much too slow to be of physiological importance

Haas, Horecker and Hogness have now reported the isolation from yeast of a new flavoprotein having a prosthetic group apparently identical with that of the old yellow enzyme (51) This flavoprotein appears to be the physiological mechanism for oxidizing CoH_2II , since it will catalyze the reduction of cytochrome *c* by CoH_2II at a very rapid rate It is likely that the enzymes previously obtained do not represent the physiological mechanism for the oxidation of reduced coenzyme II

CYTOCHROME *a*

This compound has never been isolated and is known only by its absorption spectrum (60) It has not been obtained in solution and appears to be associated with solid tissue particles On the basis of potentiometric studies Ball (9) suggested that cytochrome *a* is reduced by cytochrome *c* and therefore functions between *c* and oxygen Cytochrome oxidase has not been obtained free from cytochrome *a* However Tamiya and Ogura (110) placed cytochrome *a* before *c* in the transport scheme Keilin and Hartree (60) have obtained evidence for the existence of a new cytochrome compound which has been called a_3 and which may be identical with cytochrome oxidase However neither *a* nor a_3 were included in Figure 1 since they cannot be placed definitely

CYTOCHROME *b*

As in the case of cytochrome *a*, this compound is known only by its absorption spectrum and has not been isolated (60) On the basis of measurements of oxidation-reduction potential, Ball (9) suggested that cytochrome *b* functions as a hydrogen carrier between *c* and carriers of lower potential Lockhart and Potter (74) have pointed out that indirect evidence indicates that cytochrome *b* may be an essential link between coenzyme I and cytochrome *c* but in the case of coenzyme II it has since been shown by Haas, Horecker and Hogness (51) that it is possible to link this coenzyme directly to cytochrome *c* by means

of a new flavoprotein in the absence of *b*. The status of cytochrome *b* is therefore not known at present. Since cytochromes *a* and *b* are so unstable and since they have not been prepared free from each other or free from cytochrome oxidase, it is difficult to assign a definite role to them, although Warburg considers that all three cytochromes function in an electron transport chain which is then linked to oxygen by a fourth heme compound which is auto-oxidizable.

CYTOCHROME *c*

In contrast to cytochromes *a* and *b*, cytochrome *c* is soluble in water and has been isolated in a relatively pure form which is active in re-constructed systems (58). The cytochrome *c* obtained in the isolation process contains 0.34 per cent iron and has a molecular weight of 16,500. More recently Theorell and Åkesson (115) have proceeded further with the purification and have obtained cytochrome *c* with an iron content of 0.43 per cent. It is however, the former preparation which is generally employed in reaction mixtures. The compound has a wide distribution in animal tissues (57, 101) and can easily be shown to be oxidized and reduced by animal tissues. If a tissue preparation is strongly illuminated and examined spectrophotometrically in the absence of oxygen, the oxidation of cytochrome *c* is blocked and the reducing systems of the tissue quickly reduce the carrier, and the absorption band at 550 $m\mu$ appears. If urethane is added to block the reducing systems and oxygen is admitted, the oxidizing system functions, and the absorption band at 550 $m\mu$ disappears. Cytochrome *c* has been shown to catalytically stimulate oxidation of α -glycerophosphate and succinate (86), of glucose (52), and of the CoI linked metabolites (74, 90). Cytochrome *c* appears to be oxidized by cytochrome oxidase, since preparations of the latter which are relatively free from cytochrome *c*, do not appear to oxidize any substrate unless cytochrome *c* is added (59, 103). Furthermore chemically reduced cytochrome *c* can be oxidized by cytochrome oxidase (40, 53). However since preparations of cytochrome oxidase contain cytochrome *a* and other biocatalysts it is not possible to say that cytochrome oxidase is the sole link between cytochrome *c* and oxygen.

As for the reduction of cytochrome *c*, Warburg stated in 1938 (117) that the specific reductant of the cytochromes was unknown. Succinic and α -glycerophosphoric dehydrogenases were the only dehydrogenases from animal tissues which Ogston and Green (86) were able to link with cytochrome *c* and the coenzyme systems appeared to have no connection with the compound. This was one of the lines of evidence which led Szent-Gyorgyi to suggest that succinate plus its dehydrogenase represents the mechanism by which the coenzyme-linked systems react with cytochromic *c*. Recent work by Potter (88) however indicates that hydrogen from coenzyme I systems may reach cytochrome *c* without the action of succinic dehydrogenase, and Haas, Horecker and Hogness (51) have linked a CoII system to cytochrome *c* by means of a new flavoprotein, in the absence of cytochrome *b*.

Until cytochrome oxidase is purified to the extent that it is demonstrably free from "cytochrome-reductase" the exact mechanism of cytochrome *c* action must remain obscure but there seems little doubt that cytochrome oxidase is distinct from the reductase and that cytochrome *c* is involved in the transport of hydrogen from a great many metabolites to oxygen.

RIBOFLAVIN PHOSPHATE

This compound is also known as alloxazine phosphate, or alloxazine mononucleotide according to Warburg's system of nomenclature (117). On the basis of flavin determinations this compound and/or alloxazine adenine dinucleotide is widely distributed in animal tissues (114, 118). However, it should be pointed out that there have been no reports on the occurrence in animal tissues of any flavoproteins having this compound as the prosthetic group, and the remarks to follow have to do with yeast. It has been shown to catalytically stimulate the oxidation of reduced coenzyme II (112).

Warburg has shown (117) that the specific reductants of riboflavin phosphate are the coenzymes I and II. The flavin compound appears to be in combination with a protein of the P_2 type (Fig. 1) which also has an affinity for the reduced coenzymes, and effects the oxidation of the coenzymes and reduction of the flavin phosphate. Thus the flavin can be reduced either by stoichiometric amounts of reduced

coenzyme I or II, or by any of the metabolites which react with the coenzymes I or II, plus the appropriate dehydrogenase, plus catalytic amounts of the coenzyme

The oxidation of this carrier, in the form of the old yellow ferment takes place directly with molecular oxygen (117) Since this reaction is not cyanide sensitive, and since the oxidation of the coenzyme-requiring metabolites is sensitive to cyanide in intact tissue preparations (86), it is quite likely that the direct oxidation of the leucoflavoprotein by oxygen does not represent the physiological mechanism This discrepancy now seems to be cleared up by the isolation from yeast of a new flavoprotein having the same prosthetic group, which is able to react with cytochrome *c* at a very rapid rate (51) This is in harmony with the older observations on cyanide sensitivity and probably represents the physiological mechanism The new enzyme however is CoII specific, and an analogous enzyme for CoI has not been obtained in pure form

ALLOXAZINE ADENINE DINUCLEOTIDE

This compound was found by Straub (105, 106) and by Warburg and Christian (119) to be the coenzyme of the d amino acid oxidase of Krebs (62) which was shown by Das (29) to require a coenzyme Warburg reports its presence in all tissues so far examined, including heart, liver and kidney, as well as in Jensen sarcoma He also suggested that the compound is probably associated with other enzymes since there is no parallelism between the amount of alloxazine adenine dinucleotide and d amino acid oxidase content of various tissues

The latter idea was speedily confirmed by the finding that the coenzyme factor of Dewan and Green (30) contained alloxazine adenine dinucleotide as its prosthetic group (26), and the announcement by Ball (10) that the compound appeared to be identical with the prosthetic group of xanthine oxidase Subsequent work however made the latter conclusion less certain and Ball (11) and Corran et al (25) suggest that a higher flavin nucleotide may be involved The compound has been shown to be essential for the oxidation both of the d amino acids and the reduced coenzymes

The carrier can definitely be reduced by various d-amino acids in the

presence of the proper enzyme and in addition it is claimed by Dewan and Green (30) that either reduced CoI or CoII will reduce the compound in the presence of the appropriate protein (P_2 , Fig 1). However, Adler and Abraham report that only CoI is acted upon (1), and work by Potter (see 74) supports this latter view. As we have seen above, a new enzyme which has riboflavin phosphate as its prosthetic group, will oxidize CoII (51).

The physiological oxidant of the reduced adenine alloxazine dinucleotide in animal tissues is still unknown although it seems to be generally agreed that the cytochrome system lies between the dinucleotide and oxygen. Lockhart and Potter (74) have suggested that the present facts can be explained by assuming that the dinucleotide is oxidized by cytochrome *b*, which then is oxidized via cytochrome *c*, cytochrome oxidase and oxygen. It has not been possible to obtain an enzyme preparation in which the oxidizing systems have been separated from the reducing systems and hence requirement 5 is not fulfilled, and it is impossible to analyze completely the mechanism of action of the dinucleotide.

In the case of the d-amino acid oxidase, the dinucleotide is rapidly oxidized directly by molecular oxygen (106) but in the case of the CoI oxidizing enzyme (P_2 , Fig 1) the dinucleotide is not autooxidizable (3). The physiological importance of the d-amino acid oxidase is still unknown since the d-amino acids are not the naturally occurring type.

It is the belief of the reviewer that all of the compounds thus far considered are almost certainly important components of the hydrogen transport mechanism. We shall now consider a number of compounds which may ultimately be shown to be carriers as important as any of those listed above, but which at present seem backed by insufficient evidence to justify their inclusion in any particular part of the hydrogen transport mechanism.

VITAMIN C (ASCORBIC ACID)

This compound has been suggested as a hydrogen carrier almost since its discovery. The many papers bearing on its chemistry and physiological function were summarized by King in 1936 (61). The vitamin occurs in virtually all animal tissues in amounts compatible

with hydrogen transport function It has been obtained in pure form and has been synthesized

The reduction of dehydroascorbic acid (oxidized vitamin C) by animal tissues can be taken as an accepted fact. Schultze, Stotz and King (96) have studied the problem carefully and discuss the literature As evidence that dehydroascorbic acid can be reduced by animal tissues they point out that (a) the compound has anti ascorbic acid action and (b) its administration leads to increased excretion of ascorbic acid in man and to storage of ascorbic acid in guinea pig tissues In addition these workers showed that dehydroascorbic acid is rapidly reduced by suspensions of liver, muscle, intestine, and erythrocytes They suggested that the reduction was non-enzymic and due to direct reaction with glutathione and fixed $-SH$ compounds of the tissues, and reported that iodoacetate, arsenite and alloxan inhibited the reduction completely

King and co-workers have also studied the oxidation of ascorbic acid by animal tissues (102) They found that tissue preparations were able to catalyze the oxidation of the vitamin and concluded that the reaction was brought about by cytochrome oxidase plus cytochrome c Experiments with copper inhibitors indicated that copper was not involved They also reported that the oxidation of glutathione by a system which would oxidize vitamin C did not require the presence of the vitamin Since $-SH$ groups were the only known reductant of the vitamin the role of this compound as a hydrogen carrier remains questionable

These workers further attempted to demonstrate carrier function of vitamin C in the oxidation of dihydrocozymase, since Euler (37) mentions unpublished experiments in which the latter compound reduced dehydroascorbic acid They (95) found no reduction of the oxidized vitamin by dihydrocozymase directly, and no stimulation of a system consisting of glucose and its dehydrogenase plus coenzyme I and methylene blue However, there was no flavo protein added in these experiments and the amount present in the dehydrogenase preparation must have been very small Thus the oxygen uptake of the preparation may have been limited by the reaction between CoH_2I and methylene blue If the reaction between the vitamin and the reduced coenzyme should require the flavoprotein,

as well it may, then these experiments would not have eliminated the possibility that the oxidized vitamin can be reduced by CoII in the presence of the proper biocatalysts.

Bontoux et al. (22) and Lemberg et al. (70) have suggested that glucose dehydrogenation might be accomplished in a system in which ascorbic acid functioned as a carrier and Quastel and Wheatley (91) have reported that the oxidation of butyric and crotonic acids, but not β -hydroxybutyric acid, was maintained at a higher rate by the presence of ascorbic acid.

In spite of the vast number of papers which have dealt with this subject, we agree with Schultz, Harter and Kling (95) in their conclusion that "the concept that ascorbic acid functions in animal tissues as a respiratory carrier remains essentially unsupported by experimental evidence."

GLUTATHIONE

This compound was one of the first compounds to be considered as a hydrogen carrier and the idea has continued for some time in spite of a lack of supporting evidence.

The compound has been obtained in pure form (54) and is known to be widely distributed in animal tissues in which it is present in both the oxidized and the reduced form (43, 92).

The earlier studies have been concerned with demonstrating that glutathione can be oxidized and that it can be reduced by tissue constituents. The oxidized form can be reduced by the fixed α -SH groups of heat treated proteins (80) and also by heat labile constituents of tissue (55). However work in which both GSII and GSSC were determined (96) indicated that the content of both forms increased in an incubated tissue preparation, while the ratio between the two forms remained constant. The increase was attributed to proteolysis. Mann (78) showed that glutathione could be reduced by glucose plus glucose dehydrogenase plus a factor which probably contained the coenzymes I and II, and in 1935 Meldrum and Tarr (81) showed that glutathione could be reduced by hexose monophosphate dehydrogenase and coenzyme, and that oxygen uptake could be accomplished on the basis of the autooxidation of GSII, although the oxidation was slow.

The oxidation of pure glutathione is not catalysed by iron or copper

ions alone according to Meldrum and Dixon (80) but in addition another factor, believed to be cysteine, is required. However Lyman and Barron (77) make no mention of this. In addition to this type of oxidation, a large body of evidence has accumulated which indicates a close relation between glutathione and ascorbic acid, the former being able to reduce the oxidized form of the latter (56). This relation is complicated by the fact that glutathione is able to bind the metallic ion catalysts of ascorbic acid oxidation (18) and the fact that the concentration of the reactants profoundly affects the course of the reaction (96), which is apparently reversible. Glutathione appears to be capable of being oxidized by the cytochrome system, and apparently this reaction is independent of ascorbic acid (102).

Ogston and Green (86) tested the ability of glutathione to stimulate catalytically the oxidation of various substrates, particularly those which had been reported to reduce oxidized glutathione. In neither the glucose nor the hexose monophosphate system did the glutathione exhibit carrier function, presumably due to the sluggish autoxidation of the compound. The failure to use either flavoprotein or the cytochrome system in conjunction with glutathione in these experiments however, makes a re-examination of the question desirable. Only in the hexose diphosphate system did glutathione produce an increase in oxygen uptake and since there is no reason for supposing its rate of autoxidation would be greater in this than in other cases, the significance of this finding must be discounted. It is possible that the glutathione acted indirectly in this case by activating glyceraldehyde diphosphate dehydrogenase, the activity of which is dependent upon reduced —SH groups (94).

Probably the best test of glutathione as a carrier has been the work of Schultze, Harrer and King (95) in which a glucose oxidizing system was reconstructed from glucose dehydrogenase, coenzyme I, glutathione, ascorbic acid and nicotine hemochromogen. There was no evidence from this work that glutathione could function as a hydrogen carrier, although it has been suggested to act in just such a system on the basis of theoretical considerations (22). Nevertheless it still remains possible that the proper combination of biocatalysts necessary to demonstrate carrier function in the case of glutathione, as in the case of vitamin C, have not yet been assembled. This work has been

made more difficult by the fact that the proper total system, if it exists, probably contains at least seven or eight biocatalysts. With reference to the five criteria of hydrogen carriers listed at the beginning of this discussion, both ascorbic acid and glutathione appear to fulfill all but the fourth requirement, although critical studies on the rates of oxidation and reduction have not been made.

ADRENOCHROME

Green and Brosteaux (45) and Green (44) showed that in the lactic and malic dehydrogenase systems, additions of adrenalin stimulated oxygen uptake. In these systems, the functioning of the cytochrome system had been eliminated by the presence of the cyanide which was used to fix the oxidized metabolite formed during the reaction. In a further examination of the mechanism of the adrenalin action, Green and Richter (49) showed that adrenalin was oxidized to adrenochrome, a red pigment, and it is the latter compound which is actually responsible for hydrogen transport. This compound is apparently reduced by all CoI systems and can be oxidized either by molecular oxygen or by the cytochrome system. The turnover number (i.e. the number of oxidations and reductions per mol per minute) is very low and the rate of oxidation or reduction is therefore low. It is not possible to assess the status of this compound as a hydrogen carrier until further work has been done.

COCARBOXYLASE

This compound is the pyrophosphate of vitamin B₁ (75) and is also known as diphosphothiamine.

It has been known for some time that vitamin B₁ is widely distributed in animal tissues and that it is concerned with the metabolism of pyruvic acid (123). It seems likely that the free vitamin and the phosphorylated compound occur in animal tissues in equilibrium with each other (73), and it is now clear that the phosphorylated compound represents the active form of the vitamin (16). This is in accord with the knowledge of certain other vitamins (riboflavin, nicotinic acid) which also appear to function in the organism in the form of complex phosphate containing structures.

Work by Barron and Lyman (20) indicated that cocarboxylase is necessary for both the oxidation of pyruvic acid and its dismutation

and this is in accord with work which led Lipmann (71) to conclude that cocarboxylase is the prosthetic group of pyruvic dehydrogenase in animal tissues and certain bacteria. It is of course known that in yeast the compound appears to be the prosthetic group of the enzyme carboxylase. However as Lohmann and Schuster (75) pointed out, it is not unreasonable to assume that the compound is the prosthetic group of one protein in yeast, and of another in animal tissues.

The question of whether or not the compound functions as a hydrogen carrier in the oxidation of pyruvic acid remains far from clear, although this would seem to be a perfectly reasonable assumption. The evidence is thus far very meager. Stern and Melnick (100) have shown that the thiazole portion of the molecule can be reduced in a manner perfectly analogous to the reduction of the pyridine nucleus in coenzymes I and II. The reduced cocarboxylase thus obtained as well as the untreated cocarboxylase, are both able to cure vitamin B₁ deficiency, indicating that the body is able to reduce or oxidize the compounds fed. If the compound is reduced further than the first stage (two atoms of hydrogen per mol) the compound loses its activity, just as is the case with the pyridinium compounds. The publication of the absorption spectra of the reduced and oxidized forms of cocarboxylase (82) may make possible the elucidation of the factors which reduce and which oxidize this compound, as has been done in the case of the coenzymes I and II.

Lipmann (72) obtained a protein fraction from lactic acid bacteria which would oxidize pyruvate to acetate when both cocarboxylase and alloxazine adenine dinucleotide were added. It was suggested that the latter compound was concerned in the oxidation of the reduced cocarboxylase. This work has not been extended to animal tissues as yet.

At present it is not possible to state the mechanism by which cocarboxylase assists in the oxidation of pyruvic acid nor is it possible to state that the compound is a hydrogen carrier.

REVERSIBLY OXIDIZED METABOLITES

Fumarate and oxalacetate

According to the Szent-Györgyi theory the 4-carbon dicarboxylic acids function as hydrogen carriers in the transport of hydrogen from the tissue metabolites to cytochrome *c*. Virtually all of the facts

which have been presented in proof of the Szent-Gyorgyi theory are however equally well explained on the basis that the four carbon acids can be reversibly reduced and on the basis of the citric acid cycle theory of Krebs (66). The latter theory has been questioned by Breusch (23, 24) and by Thomas (116) but these criticisms have recently been answered by Krebs (66). The essential difference between the two theories is that the latter proposes that the 4-C acids function by condensing (in the form of oxalacetic acid) with pyruvic acid in order to bring about the oxidation of this compound by a series of reactions which include the oxidation of citric acid and the regeneration of the 4-C acids (see Fig. 4), while the former suggests that the 4-C acids function as hydrogen carriers (Fig. 2). The Szent-Gyorgyi theory has never accounted for the breakdown products of pyruvic acid or for the formation of the 4-C acids and the position now taken (16, 24) appears to be that the citric acid cycle can serve as a source of the 4-C acids, and that citrate formation can serve as a means of getting rid of any excess 4-C acids (24).

According to a recent statement of the Szent-Gyorgyi theory the mechanism of hydrogen transport is as follows (16): "H from the substrates (donators) is transferred to oxalacetate which is thus reduced to malate, from malate H is transferred to fumarate which is reduced to succinate and this, activated on the succinodehydrogenase, gives up its H to the cytochrome system" (see also 109, pp. 35-42). It was recognized by Szent-Gyorgyi (109) that coenzyme I was an intermediary between the metabolites and oxalacetate but he felt that this finding did not alter the validity of the theory. However the inclusion of the coenzyme does alter the picture considerably as is shown in Figure 2. The theoretical objections to the oxalacetate-malate part of the theory were discussed in the introduction.

The case of fumarate is considerably more obscure, mainly because succinic dehydrogenase has not been obtained as anything but a very crude preparation thus far, and hence nothing is known regarding the compounds which react directly with the succinate-fumarate pair. There is abundant evidence to cover the first four criteria listed above and it may be considered as proved that the compound is found in most animal tissues, that it can be reduced and oxidized, and that it will stimulate oxygen uptake in the tissues. The question of the existence of two systems, (the fifth requirement) has not been pre-

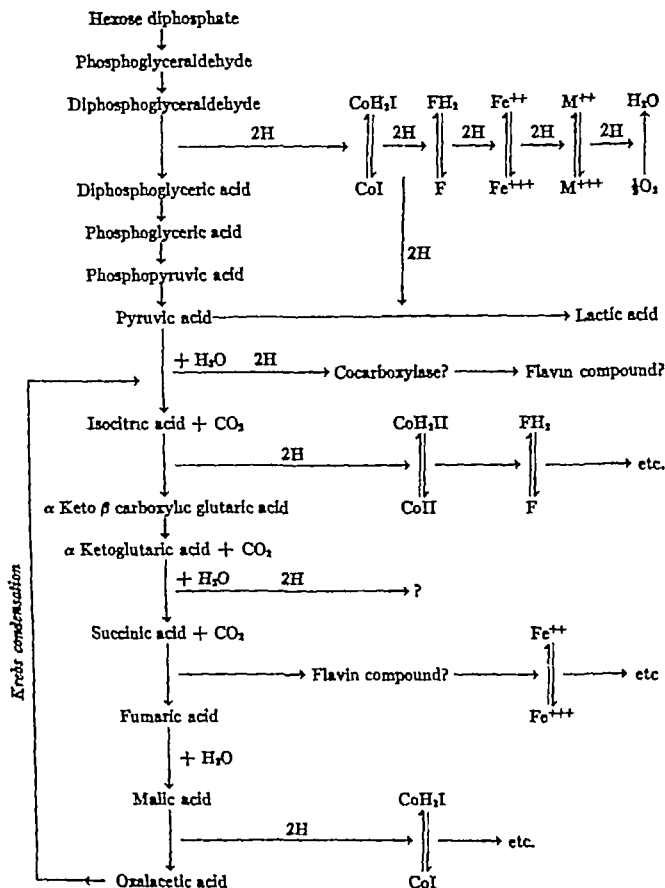


FIG. 4 CARBOHYDRATE OXIDATION AND THE CITRIC ACID CYCLE

Carriers abbreviated as in Figure 1. The complete hydrogen transport mechanism (horizontal arrows) is shown only in the case of diphosphoglyceraldehyde. The mechanisms of hydrogen transport for the remaining metabolites are indicated only so far as the stage at which they involve a carrier which is identical with a carrier in the complete scheme. The reaction indicated by the dotted line, i.e. lactic acid formation, would be expected to occur in the event of a deficiency of any of the biocatalysts between CoH₃I and oxygen or any of the biocatalysts involved in the removal of pyruvic acid, or to an inhibition of any of these biocatalysts, or to a lack of oxygen.

viously considered although Fischer and Eysenbach (41) have isolated a "fumarate hydrogenase" from yeast which has been shown to be a yellow enzyme and to utilize alloxazine adenine dinucleotide as a prosthetic group (42). Fischer doubted that this finding had any bearing on the Szent-Gyorgyi theory however, since yeast respire in the presence of malonate, oxidizes succinate slowly if at all, appears not to contain succinic dehydrogenase, and does not reduce fumaric acid in the living state. Nevertheless, if this enzyme could be isolated from animal tissues it would lend considerable support to the Szent-Gyorgyi theory.

In attempting to analyse the role of fumarate in tissue respiration there are two outstanding phenomena to be reckoned with. These are first, malonate inhibition, and second, condensation reactions.

Malonic acid has the formula $\text{COOH}-\text{CH}_2-\text{COOH}$ and since this is so similar to the structure of succinic acid, $\text{COOH}-\text{CH}_2\text{CH}_2-\text{COOH}$, both compounds are adsorbed on the enzyme. Succinic acid can be oxidized, and the resulting fumaric acid will dissociate from the enzyme, making way for more succinate. Malonate however can not be oxidized, and remains adsorbed on the enzyme, effectively blocking the action of the enzyme on succinate. It has been abundantly demonstrated that if malonate is added to a respiring preparation of pigeon breast muscle, the respiration soon comes to a complete standstill (107). Obviously if the hydrogen transport mechanisms all converge on the succinate-fumarate pair, the blocking of succinate oxidation would stop the total respiration of the muscle preparation, and this is in fact the interpretation placed upon the data by the Szent-Gyorgyi theory. There are however two other possible explanations for the malonate effect: (a) That malonate is a general inhibitor of dehydrogenases rather than a specific inhibitor for succinic dehydrogenase and (b) that malonate blocks respiration because it breaks the "citric acid cycle" at the point of the conversion of succinate to fumarate (see Fig. 4). The first possibility has been suggested by Weil-Malherbe (121) but is denied by Straub (104), who considered the malate system to be malonate insensitive. He was able to block with malonate the reduction of cytochrome *c* by malate by incubating the enzyme for 30 minutes, and concluded that hydrogen from malate reached cytochrome via the succinate-fumarate system. According

to present knowledge the hydrogen from malate must have reacted first with coenzyme I, and from this point on, the hydrogen would proceed over the same path as the hydrogen from triose phosphate (see Figs 1 and 2) The latter mechanism has been shown by Potter (88) to be malonate insensitive, suggesting that in Straub's experiments the malonate may have been acting on malic dehydrogenase. The structure of malic acid is such that competitive inhibition by malonate would not be surprising. The triose phosphate experiments (88) showed that triose phosphate dehydrogenase is not affected by malonate, since the total system was malonate insensitive. Isocitric dehydrogenase is also malonate insensitive (4). However in experiments in which malonate does inhibit the oxygen uptake it is difficult to decide whether the inhibitor acts by virtue of a blocking of succinic dehydrogenase or by action on some other component of the system. It is the opinion of the reviewer that malonate is neither a specific inhibitor for succinic dehydrogenase nor an inhibitor of dehydrogenases in general, but that it blocks by competitive inhibition only those dehydrogenases whose substrates it most closely resembles. Its greatest effect is on succinic dehydrogenase since succinate has a structure so similar to malonate, and the inhibition of other dehydrogenases, e.g. malic, is quantitatively much less. Of the two explanations alternate to the Szent-Györgyi theory the second is thus by far the more likely. The "total" inhibition of muscle respiration could be explained quite well on the basis of a break in the citric acid cycle which would result in the cessation of oxalacetate production. This would stop the oxidation of pyruvate and thus cut off all the oxygen consuming reactions in the cycle. The breaking of the cycle is mainly due to the blocking of succinic dehydrogenase but malic (28) and α ketoglutaric (121) dehydrogenases are probably affected to some extent. The fact that triose phosphate oxidation can apparently proceed in the presence of malonate, raises the question of what becomes of this reaction in malonate treated muscle. A possible explanation is as follows. The E'_0 at pH 7.2 of the lactate pyruvate system is approximately -0.181 (19) and therefore considerably more positive than the E'_0 of coenzyme I which is -0.26 at pH 7.2 according to Ball and Ramsdell (14). This means that if the oxidative removal of pyruvate is interfered with, lactate formation will compete with

oxygen consumption, and since the lactate pyruvate ratio has to be raised to 373:1 to give an E_h of -0.26 volts⁴ it would seem that the formation of lactate would greatly cut down the oxygen uptake during triose phosphate oxidation. Analytical data obtained by Greville (50) in 1937 show that lactate accumulates to some extent in malonate treated preparations from pigeon breast muscle.

The "total" inhibition of muscle respiration by malonate can therefore be explained in terms of the citric acid cycle without attributing hydrogen carrier function to succinate-fumarate. It is admitted that this explanation does not take into account the possibility of hexose monophosphate oxidation (97), but the position of this reaction in carbohydrate breakdown in animal tissues is still too obscure to be included in any integrated scheme at present.

One of the outstanding lines of evidence which favors the citric acid cycle theory as compared with the Szent-Gyorgyi theory concerns experiments on the fumarate relief of malonate inhibition. These observations cannot be satisfactorily explained by the latter theory, but can be easily explained on the basis of conversion of fumarate to oxalacetate with the subsequent condensation of the latter with pyruvic acid to give a readily oxidizable compound (66, 68, 88). In the words of Krebs (68) "The decisive argument against the view that Szent-Gyorgyi's theory fully explains the catalytic effects of fumarate is the fact that fumarate does not completely remove the malonate inhibition, *but only restores a fraction of the respiration equivalent to the amount of fumarate added*" (Reviewer's italics). In other words, when succinic dehydrogenase is blocked, fumarate behaves as a substrate and not as a catalyst. The amount of fumarate required to relieve malonate inhibition is much greater than that required for

⁴ The coenzyme system and the lactate-pyruvate system will tend to approach the equilibrium shown below (see Axelrod and Johnson in (8))

$$E'_0(\text{pyruvate-lactate}) + \frac{RT}{nF} \ln \frac{\text{pyruvate}}{\text{lactate}} = E'_0(\text{coenzyme I}) + \frac{RT}{nF} \ln \frac{\text{CoI}}{\text{CoH}_2\text{I}}$$

If $\text{CoI} : \text{CoH}_2\text{I}$ holds at 1:1 (Euler (38) reports 1:1 in normal muscle) then,

$$-0.18 + 0.0307 \log \frac{\text{pyruvate}}{\text{lactate}} = 0.26, \text{ and } \text{lactate} : \text{pyruvate} = 373:1$$

Since all of the systems are competing for hydrogen in the muscle preparation this ratio would not obtain, but since the amount of pyruvate formed would be large in comparison with the competing carriers, it should have considerable effect on the total system.

simple fumarate catalysis in the absence of malonate. This is because in the former case the oxygen uptake continues only so long as oxalacetate is available to condense with pyruvate and undergo the transformations of the citric acid cycle up to the succinate stage. When all of the fumarate (oxalacetate) has condensed with pyruvate and the condensation product has been converted to succinate the oxygen uptake virtually ceases (68). In the case of fumarate catalysis in the absence of malonate, or with low concentrations of malonate, a given small amount of fumarate will produce a much greater catalysis because it is continually reformed as the citric acid cycle functions. The fumarate relief of malonate inhibition has been given an explanation by Szent-Györgyi (107) which is based on experiments of Das (28). According to this explanation fumaric acid can be reduced to succinate in the presence of concentrations of malonate which will inhibit the oxidation of succinate to fumarate. It was then proposed that since the succinate molecule is now adsorbed on the enzyme it can be oxidized in spite of the presence of malonate. This explanation seems to require that fumarate should have a much higher affinity than succinate for the dehydrogenase. If this were true, fumarate should markedly inhibit the oxidation of succinate, and actual observations do not support the Szent-Györgyi explanation. Potter and Elvehjem (89) showed that fumaric acid had but slight effect on the oxidation of succinate, and the data of Das (28) show that fumarate inhibits succinate oxidation much less than succinate inhibits fumarate reduction. It must be remembered that the theory mentioned above was evolved to rationalize an effect for which there was at the time no other explanation. Since the data pertaining to fumarate relief of malonate inhibition are now easily explained by the condensation reaction they can not be said to prove the arguments advanced by Szent-Györgyi concerning them.

The foregoing statements apply particularly to studies made with muscle, although the citric acid cycle is also believed to function in kidney and liver. In the case of brain, there has been no successful demonstration that citric acid is an intermediary metabolite in carbohydrate breakdown, and the citric acid cycle is therefore considered not to function as such in that tissue (16, 17, 68). On the basis of fumarate stimulation and malonate inhibition of pyruvate oxidation

in brain, Banga, Ochoa, and Peters (16, 17) conclude that the hydrogen from pyruvate oxidation is brought to oxygen by means of the four carbon acids functioning as hydrogen carriers. The foregoing discussion however should have made it clear that the demonstration of stimulation by fumarate, and inhibition by malonate are not sufficient to warrant the conclusion that the 4-C acids represent the hydrogen transport mechanism in any particular reaction. It is interesting to note that Weil-Malherbe (121) reported the formation of succinic acid from pyruvic acid in brain. Since a C_4 acid could not be formed from a C_3 acid without a condensation, his work suggests that whether or not citric acid is an intermediate in this reaction, a condensation does occur, and some sort of a cyclic mechanism involving the C_4 acids is indicated.

The reviewer is not aware of any data concerning the catalytic action of the C_4 dicarboxylic acids which proves that these acids function as hydrogen carriers. In all cases the data can be satisfactorily explained without the necessity of assuming hydrogen carrier function and in some cases (fumarate relief of malonate inhibition) the data is not satisfactorily explained on the basis of hydrogen carrier function.

Krebs has been somewhat more moderate in his views, suggesting that the citric acid cycle and the hydrogen transport explanation supplement each other. Thus he states (66) "the reaction between oxalacetate and citrate suggests that the Szent-Gyorgyi system acts as a hydrogen carrier in the oxidation of citrate," and (68) "the oxidation of 'triose' to pyruvate is certainly not the only reaction in which Szent-Gyorgyi's system acts as a hydrogen carrier." While it is possible that these concepts may be true the published evidence on which Krebs bases his belief that the four carbon acids can function in the hydrogen transport mechanism all falls into the first four types of criteria listed at the beginning of this discussion, and since these can all be explained without the hydrogen transport theory there seems to be no valid reason for regarding the data as proof of the theory.

Krebs (63, 64, 65, 67, 69) has in fact suggested that certain other metabolites, in addition to fumarate can function as hydrogen carriers in animal tissues, namely, pyruvate and glutamate. His evidence (63) in the case of pyruvate and fumarate is essentially the same and will be briefly summarized. Experiments with *B. coli* illustrate the

method (63, 64) in which for the first time the quantitative aspects of criteria 2 and 3 have been worked out for reversibly oxidized metabolites. In this work it was shown that

- A Fumaric acid oxidizes glucose at the same rate as molecular oxygen
- B Succinic acid can be more rapidly oxidized by molecular oxygen than glucose.
- C Succinic acid is available in *B. coli*

Krebs then interprets these 3 points as follows (63) "On the grounds of these results the conclusion is not only permissible, but unavoidable, that fumaric acid acts as a hydrogen carrier in the oxidation of glucose because reaction 2a" (succinic acid + $O_2 \rightarrow$ fumaric acid + H_2O) "is bound to take place if succinic acid and oxygen are present, and reaction 1a" (glucose + fumarate \rightarrow succinate + CO_2) "is bound to take place if fumaric acid and glucose are present. It is further permissible to conclude that the *total* hydrogen of glucose passes, at some stage of the oxidation, through fumaric acid, and that the rate of reduction of fumaric acid is the limiting factor for the oxidation of glucose in *Bacterium coli*." The reviewer feels that the conclusions drawn are not necessarily correct. The assumption is made that reaction 1a is bound to take place aerobically, simply because it was found to take place under anaerobic conditions. This is the fundamental error which underlies the work on metabolites as hydrogen carriers, when this work is based simply on points A, B, and C, above. The work on pyruvate involves the same line of reasoning and is subject to the same criticism. If one considers the hydrogen transport mechanisms represented by the scheme in Figure 4, and considers the possibility that in the absence of oxygen various metabolites may undergo dismutations such as shown in Figure 3 via the carriers which they have in common, it becomes clear that the mere demonstration of the reduction of one metabolite by another, and of the oxidation of the reduced metabolite by oxygen, does not prove that the compound in question is a hydrogen carrier, regardless of the rates of the reactions involved. It seems likely that any two reversibly oxidizable systems will undergo anaerobic dismutation provided that there exists a third reversible system with which both can react.

Glutamic acid has also been suggested to act as a hydrogen carrier

since it can be oxidized and imino-glutaric acid can be reduced (67) but in this case the argument is strengthened somewhat by the fact that ammonia additions will stimulate the aerobic removal of a number of metabolites and further that the glutamic acid system can react with either coenzyme I or coenzyme II, thus opening up the possibility of a two-system mechanism according to the fifth criterion listed earlier

CONCLUSION

In discussing the various compounds which have been suggested as hydrogen carriers an attempt has been made to stress the need of objective experimental data in the elucidation of this problem. The alternate explanations which are represented by the citric acid cycle of Krebs and by the Szent-Gyorgyi theory for virtually identical data only serve to emphasize the need for an experimental approach which will give a decisive answer as to which is the correct explanation of the data. At present Krebs (66, 68) takes the position that the citric acid cycle does not displace the Szent-Gyorgyi theory but is supplementary to it, i.e. that fumarate functions both as a hydrogen carrier and as a source of the oxalacetate which is necessary for pyruvic acid removal. Similarly Szent-Gyorgyi suggests (109) that if the 4-carbon acids function in the citric acid cycle this function "is not *the* function but only an additional function of these C₄ dicarboxylic acids". It is entirely possible that this sort of a compromise is the correct interpretation, since it has so often been true in the past that seemingly opposing theories have been shown to be not conflicting but supplementary, with both protagonists correct in their interpretation of the limited facts at their disposal. In the present case, the reviewer, recognizing full well the probability (in the light of history) that fumarate may prove to function as a hydrogen carrier, wishes to point out that from the purely objective view point of the experimentalist, clear cut experimental data regarding the hydrogen carrier function of reversibly oxidized metabolites in general, and the Szent-Gyorgyi theory in particular, have not been forthcoming. A careful examination of a recent extensive symposium (87) on tissue respiration reveals the fact that at no session was this question clearly answered, or indeed, discussed at length.

The reviewer takes the position that as long as the data pertaining

to the Szent-Györgyi theory can be easily explained without assuming hydrogen carrier function on the part of the 4-C acids, the theory cannot be assumed to be proved. If the theory is correct, it should be possible to marshall the necessary experiments to prove it. It is felt that the facts are overwhelmingly in favor of the Krebs citric acid cycle as a working hypothesis concerning carbohydrate breakdown. Since the catalytic function of fumarate in muscle preparations can be explained on the basis of either hydrogen carrier function or condensation reactions it is desirable to study the former in the absence of the latter possibility. This can be done spectrophotometrically by observing the rate of oxidation of pure CoH I in systems with and without the fumarate succinate system. Likewise the question of malonate specificity could be studied by the same technique.

The mechanism of hydrogen transport shown in Figure 1 probably represents the main path of hydrogen transport. These carriers are probably necessary and it is fairly certain that they act in the order indicated, although the mechanism of action is obscure, the role of the 3 cytochromes is still poorly understood, and the step from the flavo-proteins to the cytochromes is not yet on a sound experimental basis. It is probable that various other carriers may link other metabolites into this transport channel at various points along the line, and alternate mechanisms may be worked out, but these remain a problem for the future. The hydrogen carriers which are involved in the oxidation of fats and amino-acids are as yet unknown, although it has been suggested (12) that the latter can be oxidized via coenzymes I and II by means of the glutamic acid system and transamination mechanisms.

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THE PATHOGENESIS OF PAROXYSMAL PULMONARY EDEMA

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INTRODUCTION

Acute pulmonary edema, first described two centuries ago, is still a subject of great interest to clinicians and physiologists. At the present time acute pulmonary edema is considered by almost all clinicians as a manifestation of failure of the left ventricle, the clinical symptoms being due to back-pressure into the blood vessels of the lung. This concept originally was based on the experimental studies

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of Welch, in 1878 that author reported the development of pulmonary edema as a result of interference with the function of the left ventricle in rabbits. Indeed the back-pressure concept is not limited solely to acute pulmonary edema but has been extended to the respiratory manifestations of chronic congestive failure as well (Harrison (203)). Attempts to explain the dyspnea of chronic cardiac decompensation on the basis of back-pressure into the lungs have already been criticized (169). As experimental and clinical observations on pulmonary edema have accumulated it has become clear that the validity of the "back-pressure," or "left ventricular failure" theory of acute pulmonary edema can no longer be considered as established. Since no complete review of the more recent studies on acute pulmonary edema is available in English, it was considered desirable to make a critical analysis of the published work on this subject, and more particularly to investigate the validity of the "left-ventricular failure" theory.

HISTORY OF LUNG EDEMA

The history of lung edema is long nearly two centuries have elapsed since the first clinical description. The first experimental researches were performed nearly 90 years ago.

The multiplicity of the causes which give rise to attacks of lung edema and the different theories attempting to explain it call attention to the fact that it is one of the most interesting, and still obscure, acute syndromes.

A brief list of the chief studies on the subject will be of some interest.

- 1752 Maloët—Clinical description (244)
- 1753 Barrère and Albertini—Clinical study (165)
- 1760-1770 van Swieten (273) Sir Percival Scott (263) Clinical studies
- 1819 Laennec—Inflammatory lung edema, lung edema in patients with cardiac diseases (224)
- 1834 Andral—Acute and chronic lung edema (158)
- 1836 J Hope—Clinical description (212)
- 1846 Legendre—Inflammatory lung edema during scarlet fever, acute lung edema in patients with nephritis (230)
- 1850-1860 Devay (182), Rilliez and Barthez (261) Complete description of the clinical picture
- 1850-1860 Virchow—Research on lung edema following fat embolism (150)
- 1850-1860 Cohnheim and Lichteim—Research on lung edema after intravenous salt solution infusion (100)

- 1869 Peter—Lung edema in pregnant women (252)
- 1873 Terrillon—Lung edema following thoracentesis (275)
- 1873 Souin de la Savinière—Lung edema during pneumonia (268)
- 1877 v Frey—Experimental researches on cutting of vagus nerves (191)
- 1878 Welch—Lung edema of the rabbit either by ligature of the aorta or by compression of the left ventricle (152)
- 1878 S Mayer—Lung edema of the rabbit by brain ischemia (130)
- 1879 Huchard—Clinical study on the relationship between lung edema and aortic lesions (213)
- 1880-1896 v Basch (86), Loewit (124), Grossmann (108), Winkler (154),
—Experimental research on toxic lung edema
- 1888 Sahli—Research on experimental edema by ligature of the aorta and by compression of the left ventricle (145)
- 1892 Alexandrow—Experimental research on mechanical lung edema (80)
- 1897 Huchard and Claude—Experimental researches on lung edema by adrenalin (111)
- 1900 Stucker—Clinical study on lung edema (271)
- 1900 International meeting in Paris on lung edema (v Basch, Teissier, Huchard, Potain (274))
- 1901 Teissier and Guinard—Experimental research on lung edema (149)
- 1906 Joreš—Research on nervous lung edema (113)
- 1908-1912 Josué (114), Cavina (97)—Experimental lung edema by epinephrine
- 1909 Miller and Matthews—Experimental lung edema (136)
- 1912 Barringer—Acute lung edema during pregnancy and labor (166)
- 1910-1912 Lian (233) and Vaquez (279)—Complete formation of the theory lung edema as consequence of left ventricular failure
- 1913 Kotowschtschikow—Research on mechanical and toxic lung edema of the rabbit and dog (117)
- 1918-1927 Moutier (248), Antonini and Biancalani (159)—Acute lung edema by injuries on the skull
- 1923-1933 Gallavardin (137), Saloz and Frommel (262), Doumer (183), Hess (208), Schellong (264) Acute lung edema in patients with mitral stenosis
- 1923-1926 Barry (85), Anrep and Bulatao (4), Lambert and Gremels (119) Lung edema in heart lung preparations
- 1928 Luisada—Experimental research on epinephrine lung edema and on the therapeutic value of anesthetics and sedatives (125)
- 1929 Glass (107), Boggian (89)—Inhibition of epinephrine lung edema by lesion of the brain and of the sympathetic system

- 1929 Antoniazzi—Lung edema by ligature of the aorta, influence of nervous stimuli and of drugs in its prevention (84)
- 1930 Luisada—Experimental lung edema by brain hypertension (126)
- 1930-1931 Frugoni (with Melli, Peserico, Luisada)—The acute lung edema A report to the Italian meeting of Int Medicine, and a book (193)
- 1929-1932 Zdansky—X-ray studies on lung edema (292, 293)
- 1931-1933 Bsteh (193), Gernez and Marchandise (199)—Clinical studies on lung edema in the nervous diseases
- 1932 Weiser—The vagal pneumonia (151)
- 1932 Luisada—Criticism of the theory of the isolated ventricular failures (monographic study) (236)
- 1931-1933 Hess—Clinical studies on the acute lung edema, lung edema after coronary thrombosis (209, 210, 211)
- 1933 Coelho and associates—Lung edema by experimental lesions of the left ventricle on the dog (98, 99)
- 1934 Reinhardt—Experimental researches on lung edema with microscopic control (142)
- 1934 S Wassermann—A monographic study on lung edema (283)
- 1934 Brunn—Luminal inhibition of experimental lung edema (92, 93)
- 1935 McGinn and White—Acute cor pulmonale (243)
- 1935 Cataldi—Lung edema by experimental lesions of the right ventricle on the dog (94)
- 1934-1938 Cataldi, Rubino—Studies on dynamic effects of the experimental lesions either of the right or of the left ventricle, and of ligature of either coronary arteries (95, 96, 143, 144)
- 1932-1938 Hochrein and associates—The lung a blood depot, vascular changes of the lung vessels (38, 39, 40, 41, 212)
- 1934-1939 Laubry and Cottet (229), Manunza (245), Astuni (161), Weissman (287) Lung edema in the diseases of the nervous system and after traumatism on the skull
- 1936 Moon and Morgan—Experimental lung edema during shock (138, 139, 247)
- 1937 Farber—Lung edema by cutting of the vagus nerves (101, 102)
- 1939 Jarisch and associates—Central lung edema by suboccipital injection of veratrin on animals (112)

I CLINICAL CONDITIONS IN WHICH ACUTE PULMONARY EDEMA OCCURS

A Heart Disease The occurrence of acute pulmonary edema in types of heart disease associated with disease of or strain on the

left ventricle mainly, is well recognized. These types include four main groups of lesions

- (a) Aortic insufficiency (due either to rheumatic or luetic involvement of the valves), arterial hypertension (either essential or symptomatic),
- (b) Mitral regurgitation, mitral stenosis (or both),
- (c) Diffuse sclerosis of the myocardium,
- (d) Coronary thrombosis

Indeed, instances of these diseases comprise the greatest number of cases of acute pulmonary edema observed clinically in adults. This fact constitutes the main clinical basis on which the "left-ventricular failure" theory rests. However the frequency of acute pulmonary edema in the above types of heart disease may very well be due to the frequency of these conditions.

It is worthy of comment that *mitral stenosis*, which gives rise to marked and prolonged increase in capillary pressure in the lungs, is not commonly associated with acute pulmonary edema, the degree of rise in intracapillary pressure is evidenced by the frequent occurrence of hemoptysis and by the marked capillary engorgement found at post-mortem examination (Parker and Weiss (251)). These findings are not in accord with a purely mechanical theory for the pathogenesis of acute pulmonary edema. On the other hand some cases observed showed that acute pulmonary edema may occur without any insufficiency of the left ventricle (Gallavardin (197), Saloz and Frommel (262), Ribierre (258), Doumer (183), Hess (208)). Of great interest and importance is the fact that acute pulmonary edema may occur in heart disease, associated either with marked *deformity of the chest* (157) (176), or with *fibrosis and silicosis of the lungs* (190). In these conditions the major strain is on the right ventricle and indeed that ventricle alone frequently shows hypertrophy. The occurrence of acute pulmonary edema in this syndrome cannot be explained on the basis of left ventricular failure.

Of importance is the pulmonary edema occurring after *coronary thrombosis*. In such cases the blood pressure often falls and the attack of edema of the lungs was considered an effect of a left ventricular failure. However, many patients with thrombosis of the left coronary artery die with acute heart failure and without edema.

of the lungs Moreover acute pulmonary edema may follow thrombosis either of the right coronary artery (Barth (163), Graser (202), Hess (210)), or of both coronary arteries (Hess (210), Sternberg (270), Leyden (231), Kohsko (222), Gallavardin (198))

Some interesting observations have been made in the treatment of attacks of pulmonary edema in heart patients Wassermann (282, 283) succeeded in ending some attacks by compressing the carotid sinus Weiss and Robb (286) obtained a marked improvement by injecting procain near one of the vagus nerves in patients with cardiac asthma

Luisada tried the intravenous injection of sedatives and anaesthetics and succeeded in stopping the attacks within a few minutes in 7 cases in which the pulmonary edema was associated with hypertension (237, 239) and later in 5 cases, of which 2 had coronary thrombosis (240) As the drugs used had, if anything, a depressant action on the myocardium, the hypothesis of left ventricular failure was not confirmed by these clinical experiments

B Diseases of the Central Nervous System Trauma to the Skull—The occurrence of pulmonary edema following skull fracture in patients with normal cardiovascular function has been known for many years Among the authors who have commented on it in recent years are Moutier (248), Antonini and Biancalani (159), Manunza (128-245), Astuni (161), Gernez and Marchandise (206), Weissman (287), Bsteh (173) Antonini and Biancalani reported it present in nearly all patients who survived more than 24 hours while Astuni reported an incidence of about 50 per cent Weissman, however, reported it present in 70 per cent of the fatal cases

Diseases of the Brain—Although acute pulmonary edema is not commonly found in diseases of the brain observed clinically, it occurs with sufficient frequency to have been reported by many observers (Moutier (248), Langeron (226), Shanahan and Ohlmacher (266), Stürtz (272), Frugoni and Luisada (193), Bsteh (173), Gernez and Marchandise (200), Laubry and Cottet (229), Lian (232), Pezza (253)) Most recently Weissman (287) recorded its presence in 70 per cent of fatal cases of intracranial hemorrhage and Farber (101, 102) reported its occurrence in a series of children with encephalitis, polioencephalitis, brain abscess and tumor, and meningitis

Manipulation of the Stellate Ganglia—Early in the history of sympathetic surgery for angina pectoris acute pulmonary edema was reported to occur during the course of manipulation of the stellate ganglia in about half the cases (Jonnesco (217, 218, 219), Danielopolu (178, 179)) More recently, probably as a result of refinement of technique, this has become uncommon

The occurrence of acute pulmonary edema in diseases of the central nervous system cannot be explained satisfactorily on the basis of left ventricular failure, since in most of the reported instances no heart disease was present.

C Diseases of the Lungs Inhalation of Toxic Gases—Acute pulmonary edema is of frequent occurrence following the inhalation of irritant gases Large numbers of cases occurred during the World War (Aschoff, Ricker, Koch, Lustig)

Pneumonia—Various types of pneumonia, especially influenzal pneumonia, may cause pulmonary edema even in young patients in whom there is no evidence of heart disease During influenza epidemics acute pulmonary edema is the cause of death not infrequently Acute infectious pulmonary edema was discussed (in this and many other diseases) by Logre (235)

Pulmonary Infarction—Even small infarcts may give rise to massive pulmonary edema (Hochrein (212)) Fat embolism following fracture is to be included in this group Occasionally, early in the course of the episode, the pulmonary edema may be limited to the side on which the infarction has occurred, spreading to involve the other lung after a period of several hours to a day Pulmonary edema following lung infarction was not found very common in acute cor pulmonale by McGinn and White (243)

Thoracentesis—Although the older literature contains references to pulmonary edema following thoracentesis, it is only occasionally seen now It is possible that refinements in technique are responsible for the disappearance of this complication (Frugoni (193))

Drowning—The fluid found in the lungs of drowned persons and animals has a high protein content (Biondi (88), Melli (134), Farber (101)) This must result from the transudation of serum into the alveoli, i.e., pulmonary edema

The occurrence of acute pulmonary edema in disorders affecting

primarily the lungs is not explainable on the basis of left ventricular failure since the strain on the heart, if any, is on the right ventricle. In some of the above listed syndromes toxic factors may be implicated.

D Uremia Acute pulmonary edema is frequently observed during the course of chronic uremia. It is especially common in patients who are receiving infusions of fluid intravenously even at rates as low as 4 to 10 drops per minute. This is usually ascribed to failure of the left ventricle although positive evidence in that regard is lacking. Moreover patients show chiefly dilatation of the right auricle and right ventricle.

E Allergy The possibility of angio-neurotic edema of the lungs occurring cannot be ruled out. In a comprehensive statistical study of allergic patients Frugoni (193) saw no proven case of this disorder, although in many cases an allergic etiology was claimed (Logre (235), Munchmeyer (249), Delamarre and Descazal (181), Day, Sisson and Vogt (180)), in some instances rather convincingly (Sticker (271)).

If allergic pulmonary edema does occur it is clear that there is nothing to suggest that left ventricular failure is at all implicated.

F Thyroid Crises Acute pulmonary edema may terminate the course of thyroid crises. Its occurrence is usually explained on the basis of left ventricular failure due to increased cardiac output, hypertension, and impaired nutrition of the myocardium.

G Beri-beri This disease frequently results in acute pulmonary edema. Since beri-beri heart is usually considered due to right heart failure, it is evident that failure of the left ventricle cannot be implicated, neuritis of the vagus nerve has been offered as a possible explanation for this type of pulmonary edema.

H Stimulation of Various Hollow Viscera The older literature contains many instances of pulmonary edema associated with distension of some abdominal organ, usually the stomach. More recent cases are that of Hochrein (212) (stomach) and that of Vigi (280) (esophagus).

These cases are hard to evaluate, either because of inadequate study or incompletely presented data, or because the patients were old people with coronary sclerosis, valvular defects or hypertension. It is probable that rapid distension of a hollow viscus does not by itself

cause pulmonary edema, though the importance of nervous reflexes suggests itself

In rare instances, sudden emptying of a distended bladder in patients with prostatic obstruction may apparently result in the development of acute pulmonary edema (see Frugoni (193)) This phenomenon is difficult to evaluate, since often the patients in whom it occurs have marked coronary sclerosis, hypertension, or both

I. Pregnancy A number of authors during the last century stressed the relationship between pregnancy and pulmonary edema in some cases Since patients developing this complication have valvular heart disease, or hypertension associated with toxemia, this type of pulmonary edema is best considered together with that due to heart disease

It is evident from the above clinical considerations, that the occurrence of pulmonary edema in many syndromes cannot be satisfactorily explained on the basis of left ventricular failure The almost universal acceptance of the "left ventricular failure" theory by clinicians is possibly to be explained by the fact that, in adults at least, by far the greatest number of cases of pulmonary edema are those associated with disease mainly of the left ventricle or with the hypertension or both. Nevertheless, the fact that numerous instances exist in which left ventricular failure cannot be used to explain pulmonary edema, strongly suggests that other factors also operate

II THE CARDIOVASCULAR DYNAMICS IN MAN DURING ACUTE PULMONARY EDEMA

It is almost impossible to obtain studies on the cardiovascular dynamics during acute pulmonary edema Attacks are frequently of short duration, or if sufficiently prolonged to permit making accurate observations, make the patient extremely uncomfortable and even desperately ill Nevertheless valuable data can be obtained from observations made in milder forms of paroxysmal cardiac dyspnea, including cardiac asthma, which are closely related to the syndrome of acute pulmonary edema itself

Measurements of *pulse rate* and *blood pressure* are easily obtained during attacks of pulmonary edema or cardiac asthma In most instances both are elevated during the attack, unless it is precipitated

by coronary thrombosis, in which case the blood pressure may be diminished (in a case of personal observation, however, in spite of the coronary thrombosis, the attack of pulmonary edema was accompanied by relative hypertension) The rise in blood pressure observed during attacks of pulmonary edema is not in harmony with the concept that the syndrome is due to left ventricular weakness, this should cause a decrease in arterial blood pressure However spasm of the arterioles due to anoxemia, reflexes, or a discharge of epinephrine have been invoked as explanations for this phenomenon If attacks of pulmonary edema are prolonged, the arterial blood pressure falls below normal and the patient may go into shock This usually follows the coughing up of very large amounts of fluid from the lungs and is probably due to markedly diminished blood volume

The venous pressure has been measured by Weiss and Robb (286) and by Altschule (156) in patients with cardiac asthma and a considerable increase was found in some Altschule suggested that this was due to a rise in intrapleural pressure associated with loss of elasticity and distensibility of the lungs (Christie and Meakins (175)) and with increased respiratory effort (Prinzmetal and associates (257)) Altschule (156) also advanced the hypothesis that this phenomenon might be important in terminating some attacks, the rise in intrapleural pressure impounding blood in the peripheral veins in a manner similar to tourniquets on the extremities In shock associated with pulmonary edema the veins are collapsed and the venous pressure is very low

Studies of the vital capacity by Weiss and Robb (286) reveal a considerable decrease during the attacks of paroxysmal dyspnea The pulmonary circulation time is also slowed and the arterial blood oxygen saturation decreases These observations explain the dyspnea observed during attacks

Measurements of the cardiac output in cardiac asthma are few Many years ago Eppinger and his co-workers (185, 186) made such a study but the method used is now discredited Lauter (227) using the Fick principle and cardiac puncture found in three cases a diminished minute volume blood flow through the lungs, i e, decreased output of the right ventricle, during attacks Weiss and Robb (286) used a dye method to measure the output of the left ventricle This

method must be considered far less satisfactory than that employed by Lauter (227). However, in a large series of cases, they found low or normal cardiac outputs in cardiac asthma.

Studies of the cardiovascular dynamics in clinical cases of cardiac asthma and pulmonary edema in man throw no light on the mechanism of its occurrence. It is therefore necessary to turn to experimental studies in animals for data bearing on this point.

III PULMONARY EDEMA IN EXPERIMENTAL STUDIES ON ANIMALS

A Heart-Lung Preparation The earliest observations by Starling and his co-workers on the heart-lung preparation record the spontaneous occurrence of pulmonary edema in such preparations. Evans (23) pointed out that this phenomenon occurred much more frequently and rapidly in cat than in dog preparations. Matsuoka (58) studied the factors responsible for its occurrence and found that increased cardiac work was not a cause but rather that prolonged duration of the experiments "and general condition of the animal and the operation" were to be implicated. He did, however, note the fact that increased pressure and passive dilation in the pulmonary circuit favored the development of edema. Barry (85), Lambert and Gremels (119), and Newton (140) concluded that the chief factor responsible for pulmonary edema in the heart-lung preparation was some change in the composition of the perfusing blood. Indeed Lambert and Gremels described degenerative changes in the pulmonary vascular endothelium due to the action of this hypothetical toxin. Raising the arterial resistance, according to Matsuoka and Barry favored the appearance of edema and Newton reported that obstructing the outflow from the lungs also favored it. Barry, however, found that venous obstruction caused only congestion unless the blood were greatly diluted, under which circumstances edema might develop. Anrep and Bulatao (4) reported pulmonary edema when either ventricle failed.

On the basis of observations on the heart-lung preparation it may be concluded that pulmonary edema is associated with failure of either ventricle and that in that type of experiment, the chief factor was a chemical change in the blood. These findings can have little bearing on the pulmonary edema observed clinically in man, except

possibly in uremia. It is important to note, however, that mechanical factors, i.e. increased cardiac work, pulmonary stasis, and dilution of the blood favor its occurrence when the tendency toward it exists. This fact is of clinical importance and will be commented on at greater length in another portion of this review.

B Increased Strain on the Left Ventricle Friedlander (106) in 1873 found that ligating the aorta proximal to the innominate artery frequently caused pulmonary edema in the rabbit. Welch (152) in 1878 performed similar experiments in rabbits with the exception that the ligature was placed distal to the left subclavian artery and two of the three great vessels coming off the arch were also tied off, pulmonary edema occurred in these animals. These results could not, however, be obtained in dogs by Sahli (145), and only in some experiments on dogs by Kotowschtschikow (117). More recently my co-worker Antoniazzi (84) repeated Welch's experiments on rabbits and only occasionally obtained pulmonary edema, unless the aorta was ligated without opening the thorax and without anesthesia. On the contrary ligature of the aorta with closed thorax caused pulmonary edema in all experiments. He observed further that either previous injection of some sedatives and anesthetics (the same found useful by Luisada in adrenalin lung edema) or stellate ganglionectomy (found effective by Boggian in adrenalin lung edema) prevented the formation of pulmonary edema. The fact that the rather drastic experiments described above caused pulmonary edema much more frequently in rabbits is particularly interesting. Dogs (owing to a more differentiated vascular apparatus in the liver and to a different balance of the autonomic nervous system—see later) have more often a tremendous dilatation of the liver, probably acting as application of tourniquets in man in reducing the circulating blood. In some of the experiments there was a serious interference with the blood supply of the brain, the significance of this in the production of pulmonary edema is discussed below. The experiments of Welch are also open to objection in that the pulmonary edema was clearly an agonal phenomenon.

It must be concluded that left ventricular strain is not proved as the sole factor in the rather inconstantly produced pulmonary edema in the above experiments.

C Interference with Left Ventricular Function By means of manual compression and the use of clamps Welch (152) in his much quoted experiments on rabbits produced pulmonary edema in some animals. These and similar experiments were repeated by Löwit (124), Alexandrow (80), Sahl (145), and Kotowschtschikow (117) with inconstant results in rabbits and only rarely positive results in dogs. The last named authors concluded that Welch's results represented agonal changes. Indeed from his protocols it would seem that in some of his experiments, the pulmonary edema appeared some time after the right ventricle had also failed.

Montanari (137) improved the technique, cutting down the size of the ventricle in contact with the circulation in rabbits by means of sutures. His animals survived without respiratory difficulty unless more than two thirds of the ventricle was excluded, in which case the animals died rapidly, only rarely showing pulmonary edema. A somewhat different and more nearly physiological approach was that of Coelho and Rocheta (98). These authors injected silver nitrate or alcohol into the wall of the left ventricle in dogs causing pulmonary edema in a large percentage of animals. The positive significance of these striking and apparently conclusive findings was negated by the observations of my co worker, Cataldi (94). That author confirmed the results of Coelho and Rocheta, but obtained the same results (with slightly lower percentage) by injecting the irritating substances into the right ventricle alone. After injection of either ventricle he noted the death of some dogs in ventricular fibrillation without pulmonary edema. Cataldi (95, 96) also studied the dynamics of the circulation in his experiments and found hypertension in the pulmonary artery and some decrease in systemic arterial pressure in animals with pulmonary edema irrespective of whether the edema of the lungs was due to a right or left ventricular lesion.

Attempts were made by Cataldi (95, 96) and by my associate Rubino (143, 144) to precipitate pulmonary edema in the dog by means of ligature of either the right or left coronary artery, but no positive results were obtained. However, the effects on the cardiovascular dynamics of ligature of either artery were the same, i.e. a slow decrease in pressure in both pulmonary artery and aorta,

and swelling of the liver. These authors concluded (1) that it is impossible to produce isolated insufficiency of one ventricle, (2) coronary occlusion causes insufficiency of the whole heart, (3) injection of irritating substances into the walls of either ventricle gives rise to pulmonary edema which must be reflex in origin, and (4) the dynamic effects on the greater and lesser circulations are the same no matter which ventricle is the site of the lesion due to injection.

Although the evidence of the above experimental studies is against the mechanical concept of left ventricular failure as a cause of pulmonary edema, the reflex nature of the processes involved cannot be considered established until studies involving complete and various types of partial denervation of the heart have been performed. Nevertheless, the above described evidence is strongly suggestive.

D Obstruction of Pulmonary Veins Attempts have been made to induce pulmonary edema mechanically by obstructing the pulmonary veins. Welch (152) claimed to have observed its occurrence in his experiments on rabbits, but Löwit (124) and Antoniazzi (83, 84) could not confirm his findings. Recently Altschule (81) used a somewhat different approach to the same problem. He introduced a balloon in the left auricle through the auricular appendage and connected it to a rubber tube running out through the chest wall. After the chest wall was sewed up the preparation consisted essentially of an intact animal (under light anesthesia, but breathing normally) with a balloon in the left auricle. Inflation of the balloon in the auricle failed to produce pulmonary edema even though inflation lasted in some experiments until the animal died after several hours.

These procedures, which must raise the pulmonary venous and capillary pressures markedly, do not cause pulmonary edema, this fact cannot be reconciled with the back-pressure theory of pulmonary edema.

E Lesions of the Central Nervous System von Frey (105) first described the development of pulmonary edema following vagotomy in 1867. Since then a number of other authors have observed the same phenomenon. These include Wolf (155), Schiff (147), Schafer (146), Weiser (151), and most recently, Farber (101, 102). Kraus (118), Brunn (92, 93), and Farber (101, 102) found that pulmonary edema could be precipitated in vagotomized animals with great ease.

by the injection of fluids intravenously, Brunn claimed to have inhibited this phenomenon by administering phenobarbital. The rôle of intravenous injection of fluids in producing edema of the lungs will be discussed in another portion of this review.

Farber (101, 102) studied the pathogenesis of pulmonary edema due to interference with the nerves to the lungs and found that the paralysis of the vocal cords is not a cause of the pulmonary edema, the real cause of it being the loss of the innervation of the lungs.

The fact that the sympathetic fibers are still intact was claimed, however, as the real cause by Jansch, because removing the vagal impulses prevented the counteracting of the effects of those of the sympathetic nerves.

Other lesions of the central nervous system may produce edema of the lungs. Thus, Brown-Séquard (90) found that stimulating the stellate ganglia caused pulmonary edema. Pulmonary edema can be induced by the injection of epinephrine as will be pointed out below. Joreš (113) reported the occurrence of pulmonary edema after electrical stimulation of the hilus of the lung, or the peripheral end of the cut vagus nerve, but Kotowtschikow (117) was unable to confirm his findings. However, when Teissier and Guinard (149) combined stimulation of the peripheral end of the cut vagus nerve with increase in intrapulmonary pressure, they claimed to have caused the development of pulmonary edema.

A recent study of Jansch, Richter and Thoma (112) showed further that it is possible to provoke acute pulmonary edema on rabbits, rats, and guinea pigs by suboccipital injection of veratrin. The edema may be prevented by injection of some anesthetics (chloral hydrate and urethane), not by others, may be prevented by large doses of atropine, but not by cutting of the vagus. The animals show arterial hypertension, strong and regular beating heart, and many signs of central excitation.

Interference with the blood supply to the brain results in pulmonary edema. Mayer (130) and Meurers (135) induced it by ligating both carotid arteries below the carotid sinus. Actually in Welch's (152) experiments on ligation of the aorta and its branches, similar changes occurred in the brain. However it is not possible to state that interference with cerebral function is the sole cause of the

edema of the lungs observed. The procedures used in this type of experiment cause a considerable elevation of the aortic pressure (due to a reflex caused by the low pressure on the carotid sinus (Heymans (37), Meurers (135))), so that the experiments are not so clean cut as they seem at first.

Luisada (126, 127) attacked the problem more directly, using a preparation in which all connections except for nervous pathways between the head and body of an animal were severed. Hypertension in the isolated head caused edema in the lungs of the isolated body.

It is clear that the action of nervous factors can produce pulmonary edema. Although, as Farber (102) showed, vasodilatation, presumably due to loss of vasomotor tone, precedes the appearance of edema, it is doubtful whether the vasodilatation alone can explain the sudden marked increase in capillary permeability which develops. Landis (48) for instance has shown that the permeability of capillaries is not increased by dilatation, but this is denied by Krogh (47). The ultimate mechanisms involved in the production of neurogenic pulmonary edema are therefore obscure. It is to be noted, however, that once the tendency toward neurogenic pulmonary edema exists, mechanical factors, such as are induced by intravenous infusions, can precipitate it.

F Intravenous Infusions Since the time of Cohnheim and Lichtheim (100, 122) attempts have been made to induce pulmonary edema by means of injections of salt solutions of various concentration. These authors could not induce it regularly with large amounts of normal saline. Hallion and Canon (110) claimed to produce edema of the lungs regularly with intravenous infusions of concentrated sodium chloride solution, but Melli and Tasso (133, 134) could not confirm these findings. More recently Warthin (281) failed to induce pulmonary edema with injections of large amounts of physiological saline solution intravenously in dogs. On the other hand, following bilateral vagotomy, edema of the lungs can be precipitated regularly by means of intravenous infusions (Kraus, Brunn, Farber).

It may be concluded that the administration of fluids intravenously in large quantities will not induce pulmonary edema unless a tendency toward it already exists because of the action of other factors.

G Injection of Epinephrine It has been known for many years that the injection of epinephrine intravenously in rabbits induces pulmonary edema (Huchard and Claude (111), Josué (114), Cavina (97)) Since pulmonary edema can be induced in this manner only in rabbits (among the usual laboratory animals), it is disputable if this phenomenon can be related to the clinical syndrome in man.² It is, however, a useful method for inducing pulmonary edema easily in large groups of animals so as to study the pathological physiology of the process as well as the effects of therapy At first sight the effects of epinephrine on the blood pressure and cardiac work would seem to favor the explanation that the edema of the lungs is due to failure of the left ventricle due to a greatly increased strain on it. However, the observations of the author (125), confirmed by Glass (107), Boggian (89) and Reinhardt (142) that some sedatives and narcotics (even without inducing anesthesia), diminish or prevent the edema of the lungs, and respiratory stimulants aggravate it, suggest that other explanations may also be valid More conclusive in this regard are other observations by Luisada (125) and by Glass (107) that cutting all communications between the brain and the periphery prevents epinephrine pulmonary edema, and that previous one-sided sympathectomy or damage to the corpora quadrigemina (107) or previous unilateral, and better, bilateral stellate ganglionectomy (89) also prevent its occurrence

These findings suggest that neural mechanisms are involved in the genesis of epinephrine pulmonary edema in rabbits However, other factors may also operate, i e , increased work of the left ventricle and hypertension

H Irritating Substances in the Lungs Studies on pulmonary edema due to the inhalation of toxic or irritating substances have been performed by many authors during the last thirty years (Winternitz and Lambert (155), Mayer and Morel (129), Laqueur and De Vries Reilingh (120, 121), Biondi (88), Melli (134)) Changes in the permeability of capillaries due to the action of toxic substances is well rec

² It is necessary, however, to remember that dogs and cats have a well developed protective mechanism in the liver veins (Mautner and Pick (57)) which is underdeveloped in rabbits man seems to have an intermediate position Action of this mechanism stops the flowing of liver blood to the heart, lowering the blood pressure and dilating the liver and the visceral vessels

ognized and explain the pulmonary edema of water poisonings, and possibly pneumonia also

It has been known for many years that after the inhalation of salt or fresh water, such as occurs in drowning, edema develops, as manifested by the presence of large amounts of fluid in the lungs. Laqueur and De Vries (87) studied pulmonary edema due to inhalation of salt water and ascribed the resultant edema to the osmotic effect. Biondi (88), however, stressed the importance of the reflex set up by stimulation of the mucosa of the respiratory tract. (113) also induced pulmonary edema by stimulating the small bronchus. The osmotic effects of the inhaled water were ruled out as causes of pulmonary edema by the experiments of Pisa (132). These authors introduced a small concentrated glucose solution into a small bronchus and by means of a balloon. They claim to have reproduced bilateral pulmonary edema by this procedure, and in this case neurogenic factor as the explanation.

Although the inhalation of toxic or irritating gases causes pulmonary edema by direct damage to the capillaries, it is probable that additional factors, neurogenic in origin, also operate.

IV PULMONARY VASCULAR DYNAMICS IN ANIMALS

An extensive literature is available in the dynamics of pulmonary circulation as studied in various types of animals. This has been reviewed by Wiggers (289). The application of observations to clinical conditions is difficult to evaluate for several reasons. (a) The unphysiological nature of the animal preparation, the introduction of measuring devices into various parts of the pulmonary circulation frequently being accompanied by shock. (b) Cutting of nerves, which, though it may clarify mechanical relations, yields results which have no necessary relation to the physiology of the intact animal. (c) Use of artificial respiration, which are known to alter cardiac dynamics. (d) Differences between animal species. There is a unanimity concerning results reported by different investigators. Another source of difficulty is possibly the fact that a

studies on the pulmonary circulation are based on changes in pressure in its various portions, whereas Hochrein and associates (38, 39, 40, 41) have shown that enormous changes in the volume of the capillary bed may occur as a result of altered dynamics, with little or no change in pressure. This is probably due to the negligible resistance to dilatation of the pulmonary capillaries due to the absence of significant tissue pressure such as is found elsewhere in the body.

It is not germane to this discussion to consider in detail the results of studies on the pulmonary vascular dynamics, discussion will be limited to those observations bearing on the pathogenesis of acute pulmonary edema.

In the heart-lung preparation according to Fuchner and Starling (31), increasing the strain on the left ventricle by increasing the peripheral resistance causes a rise in pressure in the pulmonary circuit. However Anrep and Bulatao (4) criticized these experiments pointing out that the inflow into the right ventricle was not maintained at a constant level.^{*} Welch (152) and Kotowschtschukow (158) noted a proportionate rise in pulmonary arterial pressure when the aortic pressure increases following ligation of that vessel by leaving open some of the large arteries of the aortic arch. Katz and Wiggers (43) found that ligating the aorta in a relatively intact animal caused no change in pulmonary arterial pressure. On open chest experiments, however, every increase in the inflow to the right heart, and every increase in the load on the left ventricle raised the pulmonary vascular pressure (Weber (74), and many others).

Lambert and Gremels (119) found that a rise in pulmonary arterial pressure occurs in heart-lung preparations when pulmonary edema is developing, it is impossible to state whether the change in the pulmonary arterial pressure is related to cause or effect of the pulmonary edema. Cataldi (95, 96) found that the development of pulmonary edema in animals following the injection of irritating substances into the ventricular wall was also associated with a rise in pulmonary arterial pressure and a fall in systemic pressure, irrespective of whether it was the left or the right ventricle which was damaged. He attributed this result to a reflex vascular crisis oc-

^{*} The tremendous increase in the coronary flow gave an increased inflow to the right heart.

curing in the pulmonary vessels and due to stimuli coming from the heart receptors

Farber (101, 102) has described in detail the gross and microscopic changes in the lungs during the development of pulmonary edema in animals. He records the fact that frank pulmonary edema is always preceded by a period during which only intense congestion is present.

Of great clinical importance are the observations of a number of authors on pulmonary edema of diverse origin, in various types of preparations (Matsuoka, Newton, Barry, Kraus, Brunn, Farber), that whenever the tendency toward the formation of edema of the lungs is present, its development is favored by whatever causes stasis or increase in the volume of the blood in the lungs. The relation of these findings to clinical observations on the use of venesection in combating pulmonary edema is evident.

The "left-ventricular failure" theory of pulmonary edema requires insufficiency of the left ventricle and at least relatively normal function of the right. The early experiments designed to reproduce isolated failure of the left ventricle were, as discussed previously, neither clean-cut in conception nor conclusive in result.

A more extensive criticism of the theory of the right and left ventricular failure was published by the author in 1932 (236). The observations of my co-workers Rubino (143, 144) and Cataldi (94, 95, 96) on the dynamic effects of lesions in the right and left ventricles show that failure of a single ventricle does not occur. Possible explanations for the simultaneous weakening of right ventricular action when the left ventricle fails may be diminished return to the right heart due to decreased left ventricular output and/or diminished blood supply to the right coronary artery, both impairing the function of the right ventricle. Reflex factors which may alter the function of the right ventricle may also be involved, although at present little definite is known about them. As Wiggers (289) has summarized it:

"neither experimental evidence nor logic favors the idea of Welch that a dissociation of right and left ventricular output alone can cause sufficient rise of pulmonic capillary pressure to produce edema. In the first place, such dissociation rarely occurs, and then only temporarily, in

fact it is most difficult to produce by known experimental conditions. Only a few conditions are known in which the output of the left heart is less than that of the right. Experiments actually show, however, that no extreme rise of pulmonic capillary pressure occurs even under these conditions, for such an unbalance of the cardiac mechanism is promptly remedied "

The strong probability that neurogenic factors are involved in the change in the pulmonary circulation giving rise to edema of the lungs requires at least brief consideration of the pulmonary vasomotor nerves. Olkon and Minas (60) and Sternberg and Tamari (69) have shown that the capillaries in the lungs are innervated. Daly and Euler (16) by means of improvements in technique, have demonstrated strong vasomotor activity of the sympathetic nerves to the lungs, these authors explain the ambiguous results of early authors on the basis of faulty technique. Hochrein and Keller (38, 39, 40) have advanced evidence which indicates that the chief vasomotor control of the pulmonary vessels is limited largely to the smallest vessels. There is a good deal of controversy as to whether the vagus or sympathetic nerves carry constrictor or dilator fibres to the pulmonary vessels. This literature has been summarized by Wiggers (289). As W. R. Hess concludes (206) it seems that the middle arteries of the lungs are constricted, but the smallest are dilated by the sympathetic (using the word in its anatomical sense). It must be borne in mind, in addition, that the problem of acute pulmonary edema involves not only changes in the calibre of the pulmonary capillaries but also in their permeability. No data are available concerning the latter. The concept that the permeability of capillaries is under nervous control is, however, accepted by many observers, particularly to explain the localized peripheral edema seen in various neurological disorders.

No general conclusions can be drawn concerning the relation of pulmonary vascular dynamics to edema of the lungs as observed in animals except that (1) some of the changes observed are secondary to the pulmonary edema rather than its cause, (2) once the tendency toward the development of pulmonary edema is present, its occurrence can be influenced by simple mechanical changes in dynamics, (3)

mechanical factors cannot cause pulmonary edema unless neurogenic factors are also operating

V SUMMARY AND CONCLUSIONS

It has probably become increasingly clear during the course of this review that rigid conclusions as to the pathogenesis of pulmonary edema occurring in various diseases in man cannot validly be made on the basis of available data. Indeed the purpose of this review has not been to define exactly the nature of the physiological processes which cause edema of the lungs, but rather to show the inadequacy of the generally accepted theory of left ventricular failure and also to summarize observations previously made in order to delineate the nature of problems to be attacked in the future.

Some clinicians are willing to accept the importance of neurogenic mechanisms in pulmonary edema due to trauma to the skull, etc., still retaining the concept of left ventricular failure for the common type of pulmonary edema seen in heart disease. This point of view cannot be controverted entirely, but the fact that it is impossible to reproduce the latter type regularly in animals is against it. Certainly the above discussed studies on damage to the left and right ventricles as a cause of pulmonary edema, the only thoroughly acceptable ones on the heart in animals, are against the concept of left ventricular failure.

Should the neurogenic theory be accepted, two types of acute pulmonary edema still should be admitted.

(a) Pulmonary edema caused by direct excitation of nervous centers (traumatism to the skull, cerebral hemorrhage, encephalitis, surgical excitation of ganglia, experimental suboccipital injection of veratrin),

(b) Pulmonary edema caused by a reflex, the latter caused by

- 1 Stimuli occurring either in the heart (distension of the ventricles, coronary thrombosis, experimental injection of drugs in the myocardium) or in the large arteries (arterial hypertension),
- 2 Stimuli occurring either in hollow viscera (stomach, esophagus) or irritating the respiratory tract, and the lungs

The generally unsatisfactory basis for the backward failure theory of pulmonary edema strongly favors its abandonment. However

the paucity of direct evidence favoring a purely neurogenic theory of pulmonary edema also makes impossible at present its acceptance as a sole factor, although the widespread use of morphia and other nervous depressants in the treatment of edema of the lungs is in a sense acceptance of such a theory. The problems to be investigated further are not only those of the innervation of the pulmonary blood vessels but the effects of disease elsewhere in the body, principally the heart, in influencing the nerves controlling the pulmonary vessels. In addition further studies must be made concerning the rôle of nervous factors in changing the permeability of capillaries.

A conclusion of clinical importance can be drawn from the above discussed experiments and reinforced conclusions made on the basis of clinical studies. The development and course of pulmonary edema can be influenced by mechanical procedures. When a tendency toward the occurrence of pulmonary edema in the lungs is present, stasis or an increase in pulmonary blood volume favors its development, procedures which reduce pulmonary stasis and blood volume such as venesection and the application of tourniquets to the extremities, will help in edema of the lungs. In addition drugs lowering the excitability of the central nervous system, of the respiratory center, of the vegetative nerves tend to relieve edema of the lungs.

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